SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 13: 3191-3197, 2017

Jak3 is involved in CCR7-dependent migration and invasion in metastatic squamous cell carcinoma of the head and neck

ZHONGTI ZHANG[1], FAYU LIU[2], ZHENNING LI[2], DAN WANG[1], RUIWU LI[2*] and CHANGFU SUN[2*]

Departments of [1]Prosthodontics and [2]Oral and Maxillofacial Surgery, School of Stomatology, China Medical University, Shenyang, Liaoning 110002, P.R. China

Received July 30, 2015; Accepted January 6, 2017

DOI: 10.3892/ol.2017.5861

Abstract. Patients with cervical lymph node metastasis in squamous cell carcinoma of the head and neck (SCCHN) exhibit a poor prognosis and low 5-year survival rate. It has been proven that chemokine receptor 7 (CCR7) promotes cellular migration and invasion in metastatic SCCHN. In the present study, the metastatic SCCHN PCI-37B cell line was utilized to explore the role of Janus activated kinase-3 (Jak3) in the CCR7-mediated signaling pathway in metastatic SCCHN cells. It was observed that phospho-Jak3 was expressed in SCCHN tissues. In addition, when the PCI-37B cells were analyzed in response to chemokine ligand 19 (CCL19), the ligand of CCR7, at the indicated time points, the results of the present study demonstrated that CCR7 induced Jak3 activation, and inhibition of Jak3 activity using a specific inhibitor, ZM39923, significantly attenuated CCR7-induced Jak3 phosphorylation. Migration and invasion assays and immunofluorescence staining experiments demonstrated that CCL19 promoted cell migration, invasion and F-actin rearrangment in CCR7-expressing SCCHN cells partially due to the activation of the Jak3 signaling pathway. These results demonstrate that the Jak3 signaling pathway is important for the CCR7-induced malignant biological behavior of SCCHN cells.

Correspondence to: Dr Ruiwu Li or Dr Changfu Sun, Department of Oral and Maxillofacial Surgery, School of Stomatology, China Medical University, 117 Nanjing Bei Jie, Heping, Shenyang, Liaoning 110002, P.R. China
E-mail: liruiwu88@163.com
E-mail: changfusun@hotmail.com

*Contributed equally

Abbreviations: SCCHN, squamous cell carcinoma of the head and neck; Jak3, Janus activated kinase-3; CCR7, chemokine receptor 7

Key words: chemokine receptor 7, squamous cell carcinoma of the head and neck, Janus activated kinase-3

Introduction

Squamous cell carcinoma of the head and neck (SCCHN) is a common and highly malignant tumor, typically identified in the middle or late stages of the disease, with a 5-year survival rate of 30% (1). SCCHN accounts for >90% of all head and neck tumors (2). Regional cervical metastasis is one of the most significant biological behaviors of SCCHN. The extent to which the lymph nodes of various regions are involved and the sites of the cervical lymph node metastases are associated with poor patient prognosis (3,4). Demonstrating the molecular mechanism of the development of cervical metastasis is necessary in providing novel strategies of SCCHN therapy.

It is known that chemokines and chemokine receptors participate in the development of metastasis in various types of cancer tissue (5,6). Among these chemokine receptors, it has been observed that chemokine receptor 7 (CCR7) is highly expressed in metastatic lymph nodes and invasive SCCHN cells, promoting preferential lymph node metastasis (7). The interactions between CCR7 and its ligands serve a major role in the malignant metastasis process of head and neck tumors, and the upregulation of CCR7 significantly increases the migratory and invasive ability of SCCHN cells (8). Therefore, characterizing the specific role of the CCR7 signaling pathway in the malignant metastasis of SCCHN may be helpful to evaluate whether CCR7 acts as a novel target in SCCHN therapeutic strategies.

As a non-receptor protein tyrosine kinase, Janus kinase (Jak) has been demonstrated to serve an important role in regulating various cell signaling pathways. It was reported that the tyrosine phosphorylation of the members of the Janus family of kinases, including Jak1, Jak2, Jak3 and tyrosine kinase 2 (Tyk2), may be involved in the occurrence and development of prostate and breast cancer (9). Specifically, it has been demonstrated that Jak3 is involved in dendritic cell maturation and migration via the CCR7-mediated signaling pathway (10,11). However, whether Jak3 was able to be tyrosine phosphorylated upon stimulation with chemokine ligand 19 (CCL19) in SCCHN cells, and the specific role of Jak3 activation in the migration and invasion of SCCHN cells, remains unknown.

In the present study, the role of Jak3 in SCCHN migration and invasion was evaluated. The appropriate dose and duration of CCL19 (a CCR7 ligand) treatment to activate

Jak3 phosphorylation through the CCR7 signaling pathway was investigated. Furthermore, diverse methodologies were applied to examine the role of Jak3 activation on the biological behavior of SCCHN cells, including invasion and migration. In addition, the association between the expression of phospho-Jak3, lymphatic metastasis and clinical stage was investigated. The present study observed that the activation of Jak3, through the interaction of chemokine receptor CCR7 and its ligand, is significantly involved in the invasion and migration of metastatic SCCHN. These results demonstrate the role of CCR7 signaling in metastatic SCCHN and provide new therapeutic targets for SCCHN.

Materials and methods

Human tumor samples and cell lines. In total, 70 SCCHN specimens with the adjacent metastatic (or normal) lymph nodes and 10 normal human oral mucosal tissue were obtained from the Head and Neck Tumor Center, School of Stomatology, China Medical University (Shenyang, China). SCCHN classification, including primary tumors (T), regional lymph nodes (N), distant metastasis (M) and clinical stage, was determined according to the rules of the International Union Against Cancer for Head and Neck Cancer (Tumor node metastasis, TNM classification, 1997) (12). All procedures were performed in accordance with the provisions of the Declaration of Helsinki and approved by the Ethics Committee of the China Medical University. All the specimens were obtained with the consent of the patients prior to surgery and in accordance with Health Insurance Portability. Written informed consent was received from all individuals.

PCI-37B, a metastatic SCCHN cell line expressing CCR7, was donated by the University of Pittsburgh Cancer Institute (Pittsburgh, PA, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, penicillin, and streptomycin in an atmosphere of 5% CO_2 and 95% air at 37˚C. The ZM39923 inhibitor treatment at the dose used did not affect the viability as determined using the Cell Counting Kit-8 (CCK8; cat. no. C0038; Beyotime Institute of Biotechnology, Haimen, China) according to the manufacturer's protocol.

Reagents and antibodies. CCL19 and monoclonal anti-human CCR7 antibody (10 μg/ml; cat. no. MAB197) were purchased from R&D Systems (Minneapolis, MN, USA). The Jak3 inhibitor (ZM39923) and tetramethylrhodamine-labeled phalloidin were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Anti-Jak3 (cat. no. bs-2808R) and anti-phospho-Jak3 (Tyr785; cat. no. bs-3207R) were purchased from Beijing Biosynthesis Biotechnology Co., Ltd (Beijing, China).

Immunohistochemical analysis. All the tumoral and normal specimens were obtained for histology and immunohistochemistry assay according to the common protocol (13). Sections of 5 μm thickness were deparaffinized in xylene for 10 min, and subsequently rehydrated through a graded series of ethanol (100, 95 and 70%) at room temperature. The sections were immersed in 100% methanol containing 0.3% H_2O_2 to inhibit endogenous peroxidase activity for 30 min at room temperature. For antigen retrieval, sections were put in a jar filled with 10 mM sodium citrate buffer and heated for 10 min at 95˚C using a microwave oven, and subsequently cooled to room temperature. Subsequently, sections were blocked via incubation with normal goat serum (cat. no. KIT-9706, Fuzhou Maixin Biotech Co., Ltd., Fuzhou, China) for 10 min at room temperature and incubated with rabbit polyclonal anti-phospho-Jak3 antibody (dilution, 1:100) overnight at 4˚C. The sections were washed and then incubated with 50 μl undiluted biotinylated labeled secondary antibody (cat. no. KIT-9706, Fuzhou Maixin Biotech Co., Ltd.) for 1 h at room temperature, subsequent to the incubation of primary antibody. Then, following washing three times with PBS, sections were further incubated with a complex of avidin/streptavidin-peroxidase for 10 min at room temperature. Following diaminobenzidine development, the sections were then counterstained with hematoxylin for histology. Negative controls were conducted by exchange of primary antibody for PBS. Images were captured of the stained slides and they were analyzed by microscopy (Nikon Eclipse 80i; Nikon Corporation, Tokyo, Japan) at a magnification of x100. Tumors were classified according to the percentage of positive cells: Negative (-), ≤10% or no staining; weakly positive (+), 11-50%; positive (++), 51-75%; or strongly positive (+++), >75%. For each experimental condition, ≥5 randomly selected fields were analyzed.

Western blot assay. To explore whether Jak3 is phosphorylated by CCL19, the protein expression of phospho-Jak3 and Jak3 was determined using western blot analysis. PCI-37B cells were exposed to CCL19 at a concentration of 200 ng/ml for 0, 0.25, 1, 5, 10, 15 and 30 min. The cells were lysed with ice-cold RIPA lysis buffer (Beijing Dingguo Changsheng Biotechnology Co., Ltd., Beijing, China) and centrifuged at 13,400 x *g* at 4˚C, for 30 min. Using a BCA Protein assay kit, the protein concentration of the supernatants was determined. The supernatant in the aliquots, which contained equal amounts of total protein (20 μg) were denatured and electrophoresed by 10% SDS-PAGE. Separated proteins were transferred to nitrocellulose filters. The membrane was blocked for 1 h at room temperature with 5% non-fat dried milk and subsequently incubated with rabbit polyclonal anti-Jak3 and anti-phospho-Jak3 antibody (both dilutions, 1:500) at 4˚C overnight. The primary antibodies were labeled for 1 h with a horseradish peroxidase-conjugated secondary antibody (Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China). β-actin (dilution, 1:1,000; cat. no. bs-0061R; Beijing Biosynthesis Biotechnology Co., Ltd.) served as an internal control. Bands were visualized using enhanced chemiluminescence with the BeyoECL Plus kit (P0018, Beyotime Institute of Biotechnology), according to the manufacturer's protocol. Subsequent to the determination of the time point of Jak3 activation, the cells were treated with Jak3 inhibitor (ZM39923, 10 μM) for 24 h or monoclonal anti-human CCR7 antibody (10 μg/ml) was used as an effective CCR7 inhibitor for 4 h at 37˚C. Subsequent to the cells being administrated with CCL19 for 5 min, the cells were harvested and subjected to western blot analysis, as described above, to determine Jak3 and Phospho-Jak3 protein expression.

Wound healing assay. Wound-healing assays were performed to investigate the cellular migration ability by measuring cell movement into a scraped cell-free area. The study groups consisted of control cells, cells treated with CCL19 alone and cells pretreated with ZM39923 for 24 h followed by CCL19. Cells were grown in 24-well plates and a cell-free area was created using a pipette tip. Wounded monolayers were washed with serum-free DMEM (Hyclone; GE Healthcare Life Sciences; Logan, UT, USA). Wound closure was observed after 24 h and images were captured using a microscope (Nikon TE2000-S Eclipse; Nikon Corporation) at magnification, x100. The result was calculated as the percentage of the remaining cell-free space in comparison with the initial wound space at 0 h. The experiments were performed in triplicate and between 4 and 5 scratches/well were analyzed.

Transwell and Matrigel assay. Transwell filter insert chambers (24 chambers with 8 μm pore size) were used to evaluate the biological behavior of PCI-37B cellular migration and invasion. The cell suspensions ($2x10^5$ cell/200 μl) were added to the upper chamber. Aliquots of the corresponding reagents (anti-CCR7 antibody or ZM39923) were added to the wells. Subsequently, CCL19 (aliquots of 500 ng/ml) was added to the lower chamber. The cells were subjected to 24 h of incubation at 37˚C, and then cells in the lower well were fixed with ice-cold methanol for 30 min at room temperature, then stained with 0.1% crystal violet. For the cellular invasion assay, the procedure was performed similarly as aforementioned, but the upper chamber was precoated with 500 ng/μl Matrigel solution (BD Biosciences, Franklin Lakes, NJ, USA). For each experimental condition, ≥5 randomly selected fields were analyzed by microscopy (Nikon TE2000-S Eclipse; Nikon Corporation, Tokyo, Japan) at a magnification of x200.

Immunofluorescence staining. The present study examined morphological changes in the actin cytoskeleton of SCCHN cells, which is required for tumor cell metastasis. PCI-37B cells were cultured in 24-well plates treated with 10 μg/ml CCR7 antibody for 4 h or 10 μM ZM39923 for 24 h at 37˚C, followed by treatment with CCL19 (final concentration of 500 ng/ml) for 30 min at 37˚C. Cells were fixed in 4% paraformaldehyde for 10 min, permeabilized using 0.1% Triton X-100 for 5 min at 37˚C, stained with rhodamine-labeled phalloidin and diluted to a final concentration of 10 μg/ml in PBS (containing 1% bovine serum albumin; R&D Systems, Inc., Minneapolis, MN, USA) for 1 h at 37˚C. Subsequently, the samples were washed 3 times for 10 min. F-actin distribution was evaluated by fluorescence microscopy at 495 nm.

Statistical analysis. All experiments were replicated ≥3 times. Data are expressed as the mean ± standard deviation. Differences were evaluated using the Student *t*-test or χ^2 test. P<0.05 was considered to indicate a statistically significant difference. All statistical analyses were performed with SPSS version 13.0 (SPSS, Inc., Chicago, IL, USA).

Results

Phospho-Jak3 is significantly expressed in tumor tissues and metastatic lymph nodes. In SCCHN tumor tissues, metastatic lymph nodes, normal oral mucosal tissues, the expression of phospho-Jak3 was investigated by immunohistochemical staining. The immunohistochemistry results revealed that the number of stained cells, expressing phospho-Jak3, was low or absent in normal oral mucosal tissues. By contrast, the staining of phospho-Jak3 presented in the cell cytoplasm in tumor cells and metastatic lymph node cells (Fig. 1). Additionally, the present study demonstrated that phospho-Jak3 expression was significantly associated with cervical lymph node metastasis and SCCHN clinical stage (P<0.01; Table I).

Jak3 phosphorylation induced by CCL19 in CCR7-expressing SCCHN cells. To investigate whether Jak3 is involved in SCCHN metastasis mediated by the interaction between CCR7 and its ligands, the expression of phosphorylated Jak3 protein was examined in the metastatic SCCHN cell line as determined by western blot assay. Firstly, the PCI-37B cells were pretreated with CCL19 for 0, 0.25, 1, 5, 10, 15 and 30 min, respectively. The results demonstrated that the expression of phospho-Jak3 protein was significantly modulated by CCL19 administration in a time-dependent manner. As presented in Fig. 2A, the increased expression of phosphorylated Jak3 protein appeared at 1 min, and the maximal expression was identified at 5 min subsequent to treating by CCL19, which indicated that Jak3 activation by CCL19 at an appropriate dose was transient and reversible in the metastatic SCCHN cell line. Additionally, the effect of CCR7 in regulating Jak3 activation treated by CCL19 in the PCI-37B cells was investigated. CCL19 induced a marked increased expression of phosphorylated Jak3 protein after 5 min administration (Fig. 2A). By contrast, the phosphorylation of the molecule was blocked by CCR7 antibody or ZM39923 compared with CCL19 alone (Fig. 2B). The action of CCL19 was counteracted when the CCR7 blocker was used, which suggested CCL19 application was associated with CCR7 activation. These findings strongly suggested that the CCL19/CCR7 mediated signaling induced phosphorylated activation of Jak3 in PCI-37B cells.

Jak3 activation promotes the migration and invasion of PCI-37B cells. To evaluate whether Jak3 phosphorylation has an effect on the biological behavior of SCCHN cells, the present study analyzed the migration and invasion capability of SCCHN cells by wound healing assay and the Transwell assay. To determine PCI-37B cell migration rate, the scratch-wound assay was performed. Cells were grown in 24-well plates in DMEM to confluence for 24 h, following the introduction of a wound by scratching. The present study demonstrated that subsequent to 24 h cells gradually migrated into the wound space along the wound edge. Compared with the control group, wound closure was significantly promoted in the PCI-37B cells upon addition of CCL19 at the 24 h (P<0.05; Fig. 3A), which exhibits that CCL19 enhanced the migration of PCI-37B cells. A special inhibitor of Jak3, ZM39923 significantly blocked the CCL19 induced wound closure rate compared with CCL19 alone (P<0.05; Fig. 3A). The wound-healing assay proved that Jak3 activates the ability of migration of SCCHN cells.

Furthermore, the migration and invasion assays validated that CCL19 significantly accelerated tumor progression by increasing the migration and invasion ability of the PCI-37B cells. Compared to CCL19 alone, PCI-37B cells pretreated

Table I. Association between phospho-Jak3 expression and clinicopathological factors of SCCHN.

Clinicopathological characteristic	No. of patients	Phospho-Jak3 expression		χ^2 test	P-value
		Positive	Negative		
Age, years					>0.05
≥60	39	21	18	0.125	
<60	31	18	13		
Tumor size					>0.05
T1/T2	55	30	25	0.142	
T3/T4	15	9	6		
Clinical stage					<0.01
I/II	33	10	23	16.339[a]	
III/IV	37	29	8		
Nodal metastasis					<0.01
No	35	12	23	13.027[a]	
Yes	35	27	8		

[a]P<0.01 the internal difference of phospho-Jak3 expression within clinicopathological characteristics. Jak3, Janus activated kinase-3.

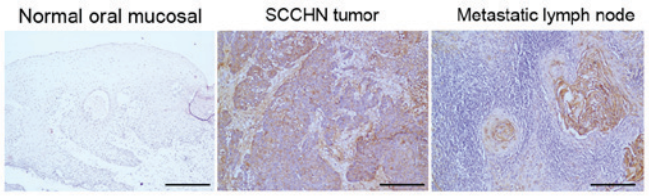

Figure 1. Expression of phospho-Jak3 was analyzed by immunohistochemistry. Representative stained images of normal oral mucosal tissues, SCCHN tissue and metastatic lymph nodes. Scale bars, 200 μm. Jak3, Janus activated kinase-3; SCCHN, squamous cell carcinoma of the head and neck.

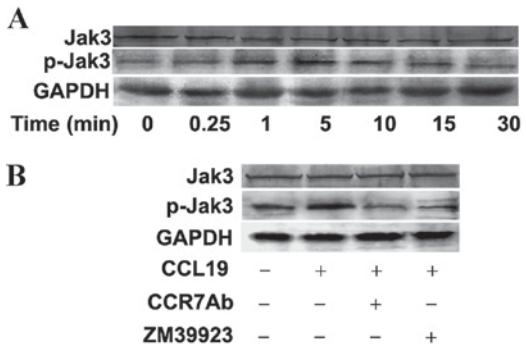

Figure 2. Jak3 phosphorylation induced by CCL19 in CCR7-expressing SCCHN cells was examined by western blot assay. (A) Time-course effect of CCL19 on Jak3 phosphorylation examined in PCI-37B cells. PCI-37B cells of control and CCL19 groups were treated with vehicle or 200 ng/ml CCL19 for 0, 0.25, 1 5, 10, 15 and 30 min, respectively. (B) Effect of CCR7 Ab and ZM39923 on CCL19-induced Jak3 activation in PCI-37B cells. PCI-37B cells pretreated with CCR7 Ab or ZM39923 followed by CCL19 were stimulated with 200 ng/ml CCL19 for 5 min. Jak3, Janus activated kinase-3; CCL19, chemokine ligand 19; CCR7, chemokine receptor 7; SCCHN, squamous cell carcinoma of the head and neck; Ab, antibody.

with CCR7 antibody followed by CCL19 resulted a significantly decrease in migration and invasion ability (P<0.05; Fig. 3B). Similarly, ZM39923 followed by CCL19 also significantly decreased the migration and invasion of PCI-37B cells compared with CCL19 alone (P<0.05; Fig. 3B). These results indicated that Jak3 performs an important role in the metastatic activity mediated through CCR7 in the metastatic SCCHN cell line.

Jak3 activation participates in F-actin rearrangement induced by CCL19 in CCR7-expressing SCCHN cells. F-actin, as the major component of cytoskeleton, performs a crucial role in tumor cell migration and motility. The rhodamine-labeled phalloidin staining demonstrated that there was a redistribution and intense impression of F-actin within the cells stimulated by CCL19. Evident pseudopodia formation was observed, which contributes to enhancing the migration and motility of PCI-37B cells. The reorganization of the F-actin was inhibited in the cells pretreated with CCR7 antibody or ZM39923, which indicated that Jak3 activation may be one of the mechanisms of F-actin polymerization induced by CCR7 and its ligands interaction (Fig. 4).

Discussion

The present experiments demonstrated that Jak3 is involved in modulating the migration and invasion induced by chemokine receptor CCR7 in metastatic SCCHN. The results of the present study may provide valuable insight into illustrating the complicated mechanisms of CCR7 signaling, through which tumor metastasis is promoted. The present study identified high expression of phospho-Jak3 in tumor tissues and metastatic lymph nodes. Phospho-Jak3 expression was associated with cervical lymph node metastasis and clinical stage of SCCHN. Additionally, the western blot analysis results demonstrated that Jak3 may be activated by CCR7 in PCI-37B cells. Furthermore, the wound healing and Transwell assays validated the hypothesis that Jak3 serves an important role

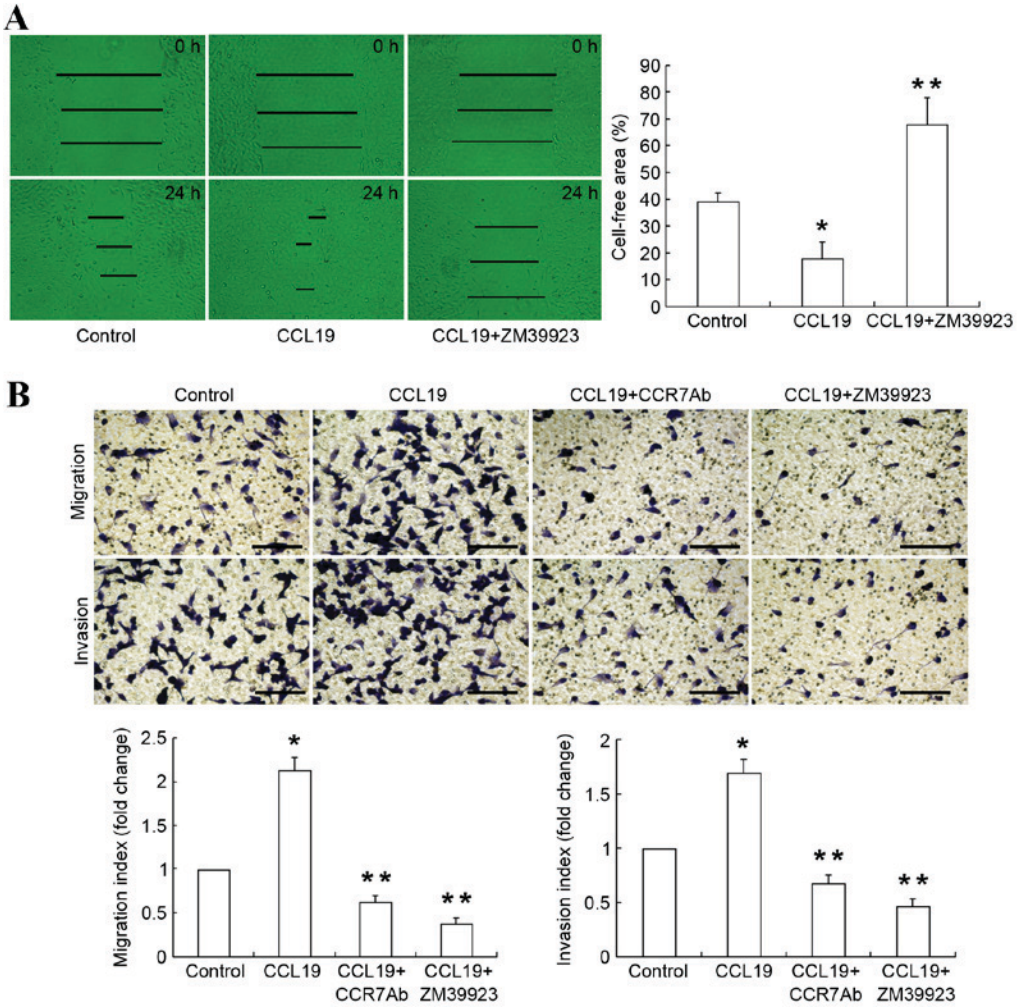

Figure 3. Jak3 activation enhances metastatic potential of SCCHN cells. (A) The cell migration rate was compared by wound healing assay. Wound closure was followed at 0 and 24 h subsequent to scratching the cell layer. (B) ZM39923 attenuated the cell migration and invasion induced by CCL19 application. Representative images and corresponding statistical data. Scale bars, 100 μm. Data are presented as the mean ± standard deviation (n=3, each group). *P<0.05 vs. the control group, **P<0.05 vs. group of CCL19 alone. SCCHN, squamous cell carcinoma of the head and neck; CCL19, chemokine ligand 19; CCR7, chemokine receptor 7.

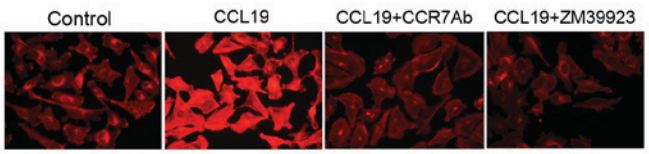

Figure 4. Jak3 inhibition blocks reorganization of the actin cytoskeleton induced by CCL19 in PCI-37B cells. The cells were stained with rhodamine-labeled phalloidin. Representative immunostaining images exhibiting control, CCL19 alone, CCR7 Ab followed by CCL19 and ZM39923 followed by CCL19 (original magnification, x400). Similar results were obtained from 3 independent experiments. Jak3, Janus activated kinase-3; CCL19, chemokine ligand 19; CCR7, chemokine receptor 7; Ab, antibody.

in CCR7-induced malignant biological behavior of SCCHN cells. Additionally, Jak3 inhibitor blocked F-actin rearrangement stimulated by CCL19 in CCR7-expressing SCCHN cells. Based on these results, the role of Jak3 was characterized in regulating the migration and invasion of SCCHN.

SCCHN, as a common malignant tumor of the head and neck, is characterized by a high degree of malignancy, high incidence of recurrence and metastasis, and high mortality. The combined treatment consisting of surgery or radiotherapy with postoperative adjuvant chemotherapy exhibits an improved outcome to prolong the overall survival in the treatment of SCCHN, but the prognosis remains far from optimistic due to the complexity and inscrutability of SCCHN (14,15). Development of novel effective therapeutic strategies is urgent to improve life quality and reduce quantity of patients with recurrent or metastatic SCCHN. The low 5-year survival of SCCHN particularly depends on the regional nodal metastasis in the neck, and thus it is crucial to explore the associated mechanisms of cervical metastasis process of SCCHN to improve outcomes for these patients (16). Numerous clinical and experimental studies identified that chemokines and chemokine receptors have significantly affected the development, invasion and metastasis of a variety of tumors, apart from performing a role in the immune system. Presently, targeted-drug therapy is a promising adjuvant treatment approach in order to produce anti-tumor effects and result in an improved prognosis for the patient (17). Studies have suggested that chemokines and chemokine receptors act as potential therapeutic targets to

inhibit tumor growth in malignant tumors (18). In particular, the CCL19/21-CCR7 signaling pathway has been associated with regional lymphatic nodal metastasis in a variety of tumors, including breast, gastric, and non-small cell lung cancer and melanoma (19,20). CCR7, as a seven-transmembrane domain G-protein-coupled receptor, presented a high expression in metastatic SCCHN and contributed to tumor progression and poor prognosis (21). Targeting of CCR7 and associated downstream molecules may control the migration and metastasis of SCCHN tumors (22). However, corresponding activation cascades of CCR7 pathway have not been accurately identified in detail in SCCHN.

The Jak family consists of 4 isoforms (Jak1, Jak2, Jak3 and Tyk2) and performs a pivotal role in the cytokine signaling pathway, modulating diverse cellular development, proliferation and differentiation (23). Among the Jaks, Jak3 that mainly exists in the lymphatic system and hematopoetic tissues, is a crucial tyrosine kinase in cell signal transduction for lymphocyte development and proliferation in the immune response (24). Jak3 is rapidly activated in phosphorylated form via cytokines by binding to their cell-surface receptors (25). Targeting Jak3-linked signal transduction pathways has shown effective immune suppression and anti-cancer effects (26,27). The activation of the Jak3 pathway participates in the proliferation of certain types of cancer including cervical cancer (28). The selective Jak3 inhibitors have been proposed as potential therapeutic reagents targeting certain cytokine-associated diseases, unlike other Jak inhibitors possessing certain unintended side effects (29,30). However, little is known regarding the role of the Jak3 signaling in SCCHN. To explore this problem, in the present study, the expression levels of Jak3 phosphorylation were detected at the indicated time points subsequent to the pretreatment of CCL19 at a dose of 200 ng/ml in a human SCCHN cell line, PCI-37B cultured in serum-free medium. The results of the western blot analysis revealed that CCL19 resulted in an increased phosphorylation of Jak3 in a time-dependent manner in PCI-37B cells. Additionally, immunohistochemical analysis demonstrated that there is an increased expression of phospho-Jak3 in tumor tissues and metastatic lymph nodes compared with the normal tissues. Additionally, the increased phospho-Jak3 expression was associated with condition of nodal metastasis and advanced stage, but not with age and tumor size ($P>0.05$). CCR7 was an independent risk factor for a higher nodal stage, recurrence and poor prognosis for SCCHN patients. Rivas-Caicedo *et al* (10) reported that Jak3 serves an important role in the cellular migration and function via the CCR7 pathway in the dendritic cell. The present study hypothesized that CCL19, by interacting with its receptor, CCR7, stimulated the activation of Jak3 in SCCHN. In the present study, PCI-37B cells were treated with or without Jak3 inhibitor ZM39923 or the CCR7 antibody followed by CCL19. The activation of Jak3 was inhibited by ZM39923 or the CCR7 antibody. As a result, the present study preliminarily proved that the interaction between CCR7 and its ligand, CCL19, enhanced Jak3 phosphorylation in the SCCHN cell line.

Previous studies have demonstrated that CCR7 expression is recognized as a key marker for predicting lymph nodal metastasis and tumor progression in tumor cells of breast and gastric cancer, as well as malignant melanoma (31-33). The authors previously investigated the role of CCR7 in tumor growth and metastasis in SCCHN (34,35). Although it was shown that Jak3 is phosphorylated by the CCL19/CCR7 pathway in SCCHN tumor, additional descriptions of the role of Jak3 in SCCHN are required. To test whether Jak3 activation was involved in CCR7-mediated migration and invasion of SCCHN cell lines, the biological behavior in SCCHN was analyzed by inhibiting Jak3 signaling using specific inhibitor ZM39923. Cell mobility and migration was detected using a wound healing assay. The cellular migration rate was increased by CCL19 application at 24 h subsequent to scratching SCCHN cell layer, suggesting that migration may be increased by CCR7 activation (13,35). These effects were blocked by the administration of ZM39923. Accordingly, the results of Transwell migration and invasion assays were consistent with the findings obtained by the wound healing assay. In addition, migration and invasion of cancer cells are functionally facilitated by actin cytoskeleton reorganization, and chemotherapeutic agents targeting the integrity of actin cytoskeleton were used to attenuate prostate cancer progression (36). In this study, an actin polymerization assay verified that ZM39923 also counteracted the effects of F-actin polymerization and pseudopodia formation as mediated by CCL19 in CCR7-expressing SCCHN cells. The alternations of cytoskeleton are the early events of migration, and are essential for the invasion and metastasis of tumor cells (37,38). Therefore, all assays emphasized the importance of Jak3 activation in the migration and invasion of SCCHN cells mediated by CCR7 signaling, which revealed one of the mechanisms for the role of CCR7 in metastatic SCCHN. Additional studies investigating the response of molecules downstream of Jak3 induced by CCR7 in SCCHN tumor cells need to be performed in the future.

In conclusion, the present study highlights the growing importance of the Jak3 signaling pathway in the metastasis of malignant head and neck tumors mediated by the interactions of chemokine receptor CCR7 and its ligands. The results of the present study also contribute to the illustration of the complicated genetic regulation mechanism of CCR7 and provide a novel target for the treatment of SCCHN.

Acknowledgements

The present study was funded with the following grants: Science Public Welfare Research Fund Projects of Liaoning Province (grant nos., 2013001017 and 2011002001); Natural Science Foundation, China (grant nos., 81201800 and 81372877); and, Shenyang Science and Technology Plan Projects China (grant no., F12-277-1-68).

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