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CCL20/CCR6 promotes the invasion and migration of thyroid cancer cells via NF-kappa B signaling-induced MMP-3 production

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ABSTRACT

CCL20, an important member of the CC-chemokine family, is the only ligand that activates CCR6. The levels of CCL20 and CCR6 are elevated in many human cancers, and CCL20/CCR6 interaction participates in the development and progression of cancer. In this present study, we found that CCR6 was overexpressed in thyroid cancer cells. Activation of CCR6 by CCL20 promoted the invasion and migration of human thyroid cancer SW1736 cells, while knockdown of CCR6 repressed the effect of CCL20. Furthermore, CCL20/CCR6 interaction induced the activation of NF-κB, and stimulated the expression and secretion of MMP-3. In addition, BAY117082, a special inhibitor of NF-κB, suppressed the expression and secretion of MMP-3 stimulated by CCL20/CCR6. Together, these results suggest that CCL20/CCR6 enhances thyroid cancer cell invasion and migration. The possible molecular mechanisms involved NF-κB activation and NF-κB-dependent MMP-3 upregulation. Thus, molecular therapies that aim at CCL20 and CCR6 may offer promising intervention strategies for thyroid cancer.

1. Introduction

Chemokines are a family of low molecular weight proteins that can be secreted by tumor, adjacent stroma and inflammatory cells, and play important roles in the occurrence and development of cancers (Ben-Baruch, 2006). It is reported that chemokines can bind to chemokine receptors, thereby affecting diverse cellular processes of tumor such as cell proliferation, apoptosis and metastasis (O'Hayre et al., 2008). As a member of the CC-chemokine family, CCL20 is the only chemokine known to interact with CC chemokine receptor 6 (CCR6) (Greaves et al., 1997). The expressions of CCL20 and CCR6 have been reported to be increased in many cancers such as colorectal and pancreatic cancers, and increased CCL20 and CCR6 expressions are closely associated with poor prognosis in patients with cancer (Frick et al., 2013; Rubie et al., 2010). Experimental data show that CCL20/CCR6 interaction increases non-small cell lung cancer cell colony-forming capacity through upregulation of IL-17 (Kirshberg et al., 2011), and enhances hepatocellular carcinoma cell growth via activation of the ERK1/2 pathway (Fujii et al., 2004). In addition, CCL20/CCR6 interaction stimulates the epithelial–mesenchymal transition (EMT) and metastasis of colorectal cancer via the PI3K/AKT-ERK1/2 signaling axis (Cheng et al., 2014).

Thyroid cancer is a common type of endocrine malignancy, with a rapidly increasing incidence in the world. Despite therapeutic strategies have been improved, the rate of death from thyroid cancer is still growing (Siegel et al., 2014). Invasion and metastasis are responsible for the majority of cancer-related death (Leber and Efferth, 2009). Reports have proved that multiple chemokines and their interaction with their receptors can stimulate tumor cell invasion and metastasis (Mukaida and Baba, 2012). However, the role of CCL20/CCR6 interaction in thyroid cancer invasion remains unclear.

Our present study here aimed to investigate the effect of CCL20/CCR6 interaction on the invasion and migration of thyroid cancer cells. We found that CCR6 expression was elevated in thyroid cancer cells. We further demonstrated that CCL20/CCR6 contributed to the invasion and migration of thyroid cancer cells. We also proved the molecular mechanisms of CCL20/CCR6 action in thyroid cancer cell invasion and migration involving NF-κB activation and MMP-3 upregulation.

2. Materials and methods

2.1. Antibodies and reagents

Antibodies against CCR6 and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against p65 and

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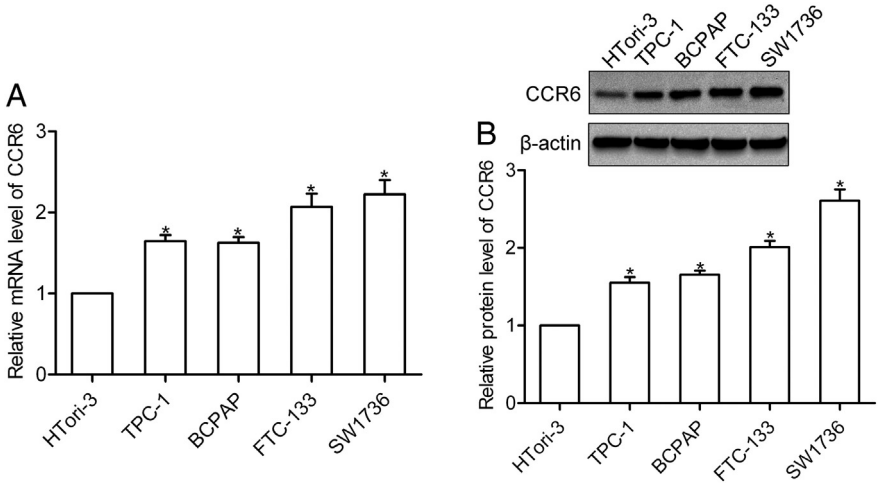

Fig. 1. CCR6 expression was upregulated in thyroid cancer cells. (A) Real-time PCR was performed to examine the mRNA expression of CCR6 in HTori-3, TPC-1, BCPAP, FTC-133 and SW1736 cells. (B) Western blotting was performed to examine the protein level of CCR6 in HTori-3, TPC-1, BCPAP, FTC-133 and SW1736 cells. β-Actin was used as an internal control. Data are presented as means ± SD of three independent experiments. *P < 0.05.

Histone H3 were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA). CCL20 was obtained from RD system (Minneapolis, MN, USA). BAY117082, a selective inhibitor of NF-κB, was obtained from Sigma (St. Louis, MO, USA), and used at 5 μM.

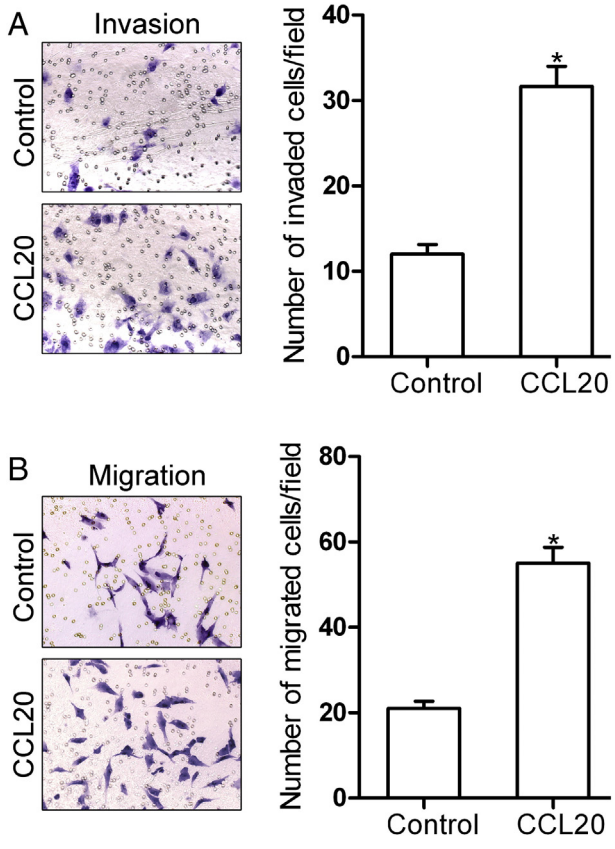

Fig. 2. CCL20 enhanced the invasion and migration of thyroid cancer cells. SW1736 cells were pretreated with or without CCL20 (10 ng/ml), and then (A) invasion assay and (B) migration assay were performed to assess the effect of CCL20 on thyroid cancer cell invasion and migration. Data are presented as means ± SD of three independent experiments. *P < 0.05.

2.2. Cell lines and cell culture

The human thyroid epithelial cell line HTori-3 was grown in RPMI 1640 medium with 10% FBS. The human thyroid cancer cell lines TPC-1, BCPAP and SW1736 were also cultured in RPMI 1640 medium with 10% FBS, while the human thyroid cancer cell line FTC-133 was cultured in DMEM medium with 10% FBS. All cell lines were obtained from American Type Culture Collection (Manassas, VA, USA), and maintained in a humidified 5% CO_2 incubator at 37 °C.

2.3. RNA interference (RNAi) assay

A CCR6 siRNA (siCCR6) and a control siRNA (siCtl) were purchased from Invitrogen (Carlsbad, CA, USA). The sequence of siCCR6 was 5′-GGGCAGAAGUUCAGAAACU-3′. SW1736 cells were plated in culture dishes and grown to 30–40% confluence before transfection. Then cells were transfected with either siCCR6 or siCtl by Lipofectamine 2000 (Invitrogen, Carlsbad, USA) and incubated for 48 h. Knockdown efficiency of CCR6 was evaluated by western blotting.

2.4. Real-time PCR

Total RNA was extracted from SW1736 cells using Trizol (Invitrogen). The amount of total RNA was measured by spectrophotometry. Then first standard cDNA was synthesized with RNA, random primer and M-MLV reverse transcriptase (Tiangen, Beijing, China). Next, real-time PCR was performed with the primers of MMP-3 and β-actin. The primers were as follows: MMP-3 forward: 5′-CGGTTCCGCC TGTCTCAAG-3′ and reverse: 5′-CGCCAAAAGTGCCTGTCTT-3′; β-actin forward: 5′-ATAGCACAGCCTGGATAGCAACGTAC-3′ and reverse: 5′-CACCTTCTACAATGAGCTGCGTGTG-3′. Relative expression changes were quantified by the $2^{-\Delta\Delta Ct}$ method.

2.5. Western blot analysis

Total protein was extracted from thyroid cancer cells by RIPA lysis buffer containing protease inhibitor cocktail (Thermo Scientific, Rockford, IL, USA). Cytoplasm and nuclear proteins were extracted from SW1736 cells by a Nuclear–Cytosol Extraction kit (Applygen Technologies Inc., Beijing, China). Protein concentrations were determined using a bicinchoninic acid assay (Applygen Technologies Inc.). Then protein of each sample (about 50 μg) was subjected to the SDS-PAGE gel and