The CCL7-CCR10 axis contributes to promoting proliferation, migration, and invasion of lung squamous cell carcinoma

Authors: Baijun Li, Caizhou Wei, Yonglong Zhong, Jianwei Huang and Rizhu Li

DOI: 10.14670/HH-18-525
Article type: ORIGINAL ARTICLE
Accepted: 2022-09-28
Epub ahead of print: 2022-09-28
The CCL27-CCR10 axis contributes to promoting proliferation, migration, and invasion of lung squamous cell carcinoma

Running title: CCL27-CCR10 affects LUSC progression

Authors: Baijun Li¹^, Caizhou Wei²^, Yonglong Zhong¹, Jianwei Huang¹, Rizhu Li³*

^ These authors contributed equally to this work

Affiliations:
1 Department of Thoracic Surgery, The People’s Hospital of Guangxi Zhuang Autonomous Region, Nanning 530021, PR China
2 Department of Respiratory, The People’s Hospital of Guangxi Zhuang Autonomous Region, Nanning 530021, PR China
3 Department of Cardiothoracic and Vascular Surgery, Affiliated Hospital of Youjiang Medical University for Nationalities, Baise 533000, PR China

*Corresponding Author: Dr. Rizhu Li, Department of Cardiothoracic and Vascular Surgery, Affiliated Hospital of Youjiang Medical University for Nationalities, No.18 Second Zhongshan Road, Youjiang District, Baise 533000, PR China. Tel: +86-13607769700, Email: li_rizhu@163.com
Abstract

Lung cancer is characterized by its high mortality and morbidity. A deep understanding of the molecular mechanisms of lung cancer tumorigenesis helps to develop novel lung cancer diagnostic and therapeutic strategies. However, the picture of the associated molecular landscape is not yet complete. As understood, chemokine-receptor interactions contribute much to lung cancer tumorigenesis, in which CCR10 also plays an important role. This study aimed to expand the knowledge of CCR10 in lung squamous cell carcinoma (LUSC) in the manner of molecular mechanism and biological functions. Using GEPIA database, the survival analysis between LUSC patients with high and low CCR10 expressions was performed, showing that CCR10 could be regarded as a risk factor for LUSC patients. Subsequently, CCR10 protein and mRNA expressions in LUSC were examined by qRT-PCR and western blot respectively. The results indicated that CCR10 was highly expressed in LUSC cells. The results of CCK-8, colony formation, and Transwell assays presented that CCL27, the ligand of CCR10, promoted proliferative, migratory, and invasive abilities of LUSC cells by activating CCR10. Also, the PI3K/AKT signaling pathway was verified as the involved pathway by western blot. Overall, it could be concluded that the CCL27-CCR10 regulatory axis can activate the PI3K/AKT pathway fostering the malignant features of LUSC cells.

Keywords: CCL27; CCR10; lung squamous cell carcinoma; PI3K/AKT pathway; progression
Introduction

Because of increasing air pollution and the prevalence of smoking, the morbidity of lung cancer is increasing globally (Ashraf-Uz-Zaman et al., 2020; Taghizadeh-Hesary and Taghizadeh-Hesary, 2020). Current therapeutic strategies for lung squamous cell carcinoma (LUSC), such as surgical resection, radiotherapy, and chemotherapy remain insufficient as the methods do not work well for advanced lung cancer patients. Additionally, with the advancement of technology, targeted therapy, immunotherapy, and cytoreduction are progressively being applied to clinical research and treatment (Pakkala and Ramalingam, 2018). Currently, immunotherapy is seen as one of the most promising frontiers for LUSC. Drugs such as Nivolumab, Pembrolizumab, Durvalumab, and Atezolizumab that are designed based on the anti-tumor immunity of lung cancer patients are widely used in second-line treatments and exert encouraging clinical efficacy (Paz-Ares et al., 2018; Kato et al., 2019; Geraci and Chablani, 2020). Drugs that target key immune-related sites such as PD-1/PD-L1, CTL4, and TIGIT are considered to have favorable efficacy, but many issues still exist in clinical treatment. For instance, a clinical trial showed that the objective response rate (ORR) was only 23% for non-small cell lung cancer (NSCLC) patients treated with Nivolumab (Gettinger et al., 2016). Despite previous assessment of drug efficacy in patients with PD-L1 immunohistochemical technique, ORR was 28% for positive patients and 14% for negative patients, demonstrating the limitation of applying immunotherapy in clinical practice (Gettinger et al., 2016). Hence, it is crucial to analyze the regulatory mechanism of the cancer-immune microenvironment as well as the function of chemokines and cytokines in the tumor microenvironment.

The CC family of chemokines (a class of chemokines containing the C-C domain in their N terminals) is a vital cytokine family that modulates various cell functions in vivo and plays a pivotal role in immunity (Korbecki et al., 2020). Recent investigations (Facciabene et al., 2011; Lanca et al., 2013; Tripathi et al., 2014) displayed that these chemokines have dual effects on the tumor microenvironment. They can repress tumor growth by recruiting tumor-infiltrating lymphocytes (TIL) or exert an oncogenic effect.
by recruiting lymphocytes such as regulatory T cells (Treg) and tumor-associated macrophages (TAM). Additionally, chemokines and their receptors can directly affect tumor cells to drive important oncogenic events, including cell proliferation, migration, and invasion. For instance, CXCL8 fosters the growth and metastasis of hepatocellular carcinoma (HCC) cells (Yin et al., 2017). CCR10, an essential cell transmembrane chemokine receptor, is involved in modulating the in vivo immune system and establishing an oncogenic cascade that promotes cancer occurrence (Simonetti et al., 2006). Relative studies on HCC, breast cancer, and melanoma reported that CCR10 and its ligands CCL27/CCL28 play a central role in modulating cell proliferation, migration, and invasion (Monteagudo et al., 2012; Lin et al., 2017; Wu et al., 2018). Based on these investigations, we speculated that CCR10 and its ligand CCL27 were key players in LUSC.

Herein, we revealed through bioinformatics analysis that CCR10 expression was implicated in the prognosis of LUSC patients. We provide experimental evidence to favor the finding by investigating the role of CCR10 and its ligand CCL27 in the occurrence of LUSC. The PI3K/AKT pathway was identified to be the key one downstream of CCL27-CCR10 to promote LUSC progression. Thus, we designed experiments to explore the relationship between CCL27-CCR10 and PI3K/AKT. Above all, our research focused on the effect of CCR10 (a key receptor of CC chemokines) and its ligand CCL27 on LUSC cells. This research may provide a reference for the exploration of tumor immune microenvironment, popularization, and application of tumor immunotherapy.

1 Materials and methods

1.1 Bioinformatics analysis

The survival analysis of CCR10 for LUSC patients was achieved by Gene Expression Profiling Interactive Analysis 2 (GEPIA 2) database (http://gepia2.cancer-pku.cn/). First, 25% of samples with the highest and lowest expression levels were excluded, and the rest of the samples were classified into high- and low-expression groups with the median value of CCR10 expression as the cut-off value (Qi et al., 2021).
The survival status of the two groups was plotted.

1.2 Cell culture

Human normal lung cell line HLF-a (BNCC100864) and human LUSC cell lines SK-MES-1 (BNCC100167), NCI-H520 (BNCC338254), NCI-H1703 (BNCC101663), and EBC-1 (BNCC341894) were accessed from BeNa Culture Collection (BNCC, China). SK-MES-1 cells were prepared in Modified Eagle’s Medium (MEM) containing 10% fetal bovine serum (FBS, Sigma, USA). The rest of the cell lines were all prepared in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Sigma, USA) with 1% double antibiotics and 10% FBS. All cells were maintained at 37 °C in an atmosphere of 5% CO$_2$-95% air. Recombinant human CCL27 and AKT inhibitor A6730 were purchased from Sigma-Aldrich (USA). CCL27 (Abcam, UK) and A6730 were added to the medium at a final concentration of 100 ng/mL and 300 ng/mL, respectively. The control group was supplemented with a corresponding volume of 1× phosphate-buffered saline (PBS, Thermo Fisher, USA).

1.3 Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was isolated from cells with RNeasy Mini Kit (Qiagen, Germany) following the manufacturer’s protocol. Then, RNA concentration was assessed by NanoDrop™ One (Thermo Fisher, USA). Next, inverse transcription was performed on RNA with QuantiTect Reverse Transcription Kit (Qiagen, Germany). The obtained complementary DNA (cDNA) was used for qRT-PCR. Two-step RT-qPCR detection of cDNA was carried out with QuantiNova SYBR® Green PCR Kit (Qiagen, Germany) following the manufacturer’s instructions. β-actin was set as endogenous control, and the 2$^{-\Delta\Delta Ct}$ method was applied to analyze the relative expression of target genes. Each experiment was set with 3 groups of biological repetition. Primer sequences of qPCR are listed as follows: CCR10: Forward: 5’-TGAAGAGGACGCATACTCGG-3’; Reverse: 5’-CCACGGTCAGGGAGACACT-3’. β-actin: Forward: 5’-TCCGGCACTACCGAGTTATC-3’; Reverse: 5’-GATCCGTTGAGCAGATCGC-3’.
1.4 Western blot

Radioimmunoprecipitation assay (RIPA) lysis buffer (Thermo Fisher, USA) was utilized to extract total proteins from cells. NanoOrange™ Protein Quantitative Kit (Thermo Fisher, USA) was implemented to quantify the isolated proteins. Total proteins were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and were transferred onto the polyvinylidene fluoride (PVDF) membrane. The membrane was blocked with 5% skim milk and incubated with primary antibodies and a secondary antibody. The primary antibodies against CCR10, PI3K (PIK3CB), AKT, p-AKT, and the corresponding HRP-labeled secondary antibodies were accessed from Invitrogen. After incubation, protein bands were visualized using an enhanced chemiluminescence reagent (Santa Cruz Biotechnology, China). Each experiment was repeated in triplicate.

1.5 Cell transfection

Sh-CCR10 was used to knock down CCR10 expression and was purchased from Origene (Origene, USA) along with its corresponding negative control (sh-NC). The plasmid transfection was conducted by Lipofectamine 2000 (Thermo Fisher, USA). 48 h later, cells were collected for the following experiments.

1.6 Cell counting kit-8 (CCK-8)

Cells were collected after transfection and inoculated into 96-well plates. According to the manufacturer’s protocol, CCK-8 (MedChemExpress, USA) kit was implemented to measure cell viability. In short, 10 µL CCK-8 solution was added to each well after cell culture for 0, 24, 48, and 72 h, followed by another 2 h of cell incubation. A microplate reader was applied to measure the optical density (OD) value at 450 nm. Three biological replicates were carried out for each experiment. The results are presented as cell viability percentage referenced by the control group.
1.7 Colony formation assay

The proliferative ability of LUSC cells was detected by colony formation assay. Cells were plated to 6-well plates at the same density. Culture plates with RPMI-1640 medium containing 1% double antibiotics and 10% FBS were recommended for cell culture. After 2 weeks of culturing, cells were fixed with 4% paraformaldehyde and stained with 0.5% crystal violet. The number of colonies was counted and recorded. Three biological replicates were carried out for each experiment.

1.8 Transwell migration and invasion assays

The transfected LUSC cells were collected, and their migratory and invasive abilities were measured by Transwell assay. To assess the migratory ability of LUSC cells, cells were inoculated to a serum-free medium in the upper chamber (8 µm pore size) of the Transwell device (Corning, USA). RPMI-1640 medium containing 10% FBS was supplemented to the lower chamber. After incubation at 37 °C for 1 day, cells that did not migrate in the upper chamber were swabbed with sterile swabs, while the migrated cells were fixed with 4% paraformaldehyde and stained with crystal violet. The number of cells in 5 random fields was calculated. As for the invasive ability of LUSC cells, 30 µL of Matrigel was covered on the upper layer before inoculation. Other steps were the same as the migration assay.

1.9 Statistical processing

All assays were repeated at least three times and the experimental data are presented as mean±standard deviation (SD). Figures were analyzed and plotted on Graphpad Prism 8 (Graphpad, USA). Multiple groups were first subjected to one-way analysis of variance (ANOVA) for the significant test of differences, followed by Student t-test for post hoc testing. For comparison between the two groups Student t-test was used. \( P<0.05 \) indicated that the difference between groups was significant.
2 Results

2.1 CCR10 is highly expressed in LUSC cells and is associated with the prognosis of LUSC patients

To confirm whether the expression of CCR10 correlates with the survival outcomes of LUSC patients, the GEPIA database was introduced to conduct K-M analysis between the high- and low-CCR10 expression groups. The result presented that the patients with high CCR10 expression suffered from relatively poor survival outcomes (Figure 1A). To examine CCR10 expression in LUSC, CCR10 mRNA expression was detected by qRT-PCR among the different LUSC cell lines and normal lung cells. As illustrated in Figure 1B, in comparison with the normal lung cell line (HLF-a), CCR10 expression was conspicuously up-regulated in LUSC cell lines, with the highest CCR10 mRNA expression in the NCI-H520 cell line. CCR10 protein expression in LUSC cells was measured by way of a western blot test. The result exhibited that CCR10 protein expression increased prominently in LUSC cells as compared to normal cells, with the highest CCR10 protein expression in NCI-H520 cells (Figure 1C). These findings demonstrated that CCR10 was highly expressed in LUSC cells.

2.2 CCL27-CCR10 facilitates the proliferation, migration, and invasion of LUSC cells

CCR10 modulates biological processes of cells through its ligands, such as CCL27, and the CCL27-CCR10 regulatory relationship is theorized to facilitate cancer progression (Kai et al., 2011). To investigate the effect of CCL27-CCR10 on LUSC cells, cellular functional assays were performed to assess the phenotype changes in LUSC cells. NCI-H520 cells were first treated with CCL27 or sh-CCR10. Transfection efficiency was detected by qRT-PCR and western blot. As indicated in Figure 2A-B, CCR10 mRNA and protein expression levels were prominently decreased in LUSC cells treated with sh-CCR10 compared to the cells treated with sh-NC, but CCR10 expression was not affected by the alteration of CCL27 concentration. Next, changes in
cell viability and proliferative ability were assessed using CCK-8 and colony formation assays, which displayed that CCL27 could enhance LUSC cell proliferative potential. Moreover, CCR10 knockdown slightly weakened the viability and proliferative ability of LUSC cells, while CCL27 was able to restore this inhibitory effect (Figure 2C-D). The migratory and invasive abilities of cancer cells were vital indicators for assessing the malignant degree of cancer. The results of Transwell migration and invasion assays revealed that after CCL27 treatment, the migratory and invasive capacities of LUSC cells were strengthened markedly, however, CCR10 knockdown suppressed these abilities, which could be rescued by concomitant transfection of CCL27 (Figure 2E). These results manifested that CCL27-CCR10 facilitated the proliferation, migration, and invasion of LUSC cells.

2.3 CCL27-CCR10 regulatory axis activates PI3K-AKT pathway

We further examined the potentially involved signaling pathways for CCL27-CCR10 tumor-promoting regulation. The PI3K/AKT pathway, downstream of CCL27-CCR10, is associated with the malignant progression of HCC (Wu et al., 2018). To identify the interaction between the CCL27-CCR10 axis and the PI3K/AKT signaling pathway in LUSC cells, the PI3K/AKT pathway-related proteins were analyzed by western blot in the constructed cell groups. As illustrated in Figure 3, adding CCL27 improved AKT phosphorylation while CCR10 knockdown weakened AKT phosphorylation. Furthermore, CCL27 restored the decreased trend caused by the CCR10 knockdown. These findings presented that CCL27-CCR10 was able to activate the PI3K/AKT pathway in LUSC cells.

2.4 CCL27 facilitates the malignant progression of LUSC cells by activating the PI3K/AKT pathway

To identify that PI3K/AKT was downstream of CCL27-induced activation, AKT inhibitor A6730 was employed in LUSC cells. The result indicated that AKT phosphorylation was up-regulated with CCL27 treatment, but it was down-regulated with A6730 treatment (Figure 4A). The results of CCK-8 and colony formation assays
illustrated that A6730 weakened the promotive effect of CCL27 on LUSC cell proliferation (Figure 4B-C). As such, the results of the Transwell assay indicated that A6730 was able to eliminate the promotive effect of CCL27 on the migration and invasion of LUSC cells (Figure 4D). Taken together, CCL27-CCR10 mediated the PI3K/AKT signaling pathway, thereby affecting the malignant progression of LUSC.

Discussion

This study dived into the role of CCR10 and its ligand CCL27 in the occurrence and progression of LUSC and demonstrated the high expression of CCR10 in LUSC cells. With regard to the CCR10 expression evaluation, some expression differences seemed to exist among the different LUSC cell lines. The expression of CCL27-CCR10 is closely related to the migration and invasion of tumor cells (Korbecki et al., 2020). Likewise, the expression of CCR10 is markedly correlated with the degree of tumor metastasis and TNM grade of patients by detecting the clinical characteristics of different tumor patients (Lin et al., 2017; Liu et al., 2021). Yonggang Liu et al. (Liu et al., 2021) also demonstrated the increased expression of CCR10 in NSCLC cells. Additionally, Hiromichi Kai et al. (Kai et al., 2011) presented that CCR10 expression is upregulated in breast cancer cells, consistent with our experimental results. For this phenomenon, we speculated the different extent of malignant degrees causing different expressions of CCR10 in cancer cells. Subsequently, we identified that CCL27-CCR10 was able to activate the PI3K/AKT pathway to facilitate cell proliferation, migration, and invasion.

Immune-related studies have always been the focal point of front-edge cancer research, and CC chemokines with dual cancer-promoting and inhibiting effects are also research hotspots (Nagarsheth et al., 2017). Bioinformatics analysis theorized that CCR10 affected outcomes of LUSC patients, and patients with high CCR10 expression were correlated with unfavorable outcomes. Additionally, it was confirmed through in vitro experiments that CCR10 was highly expressed in LUSC cells. CCR10 facilitates the proliferation and migration of various cancers. CCR10 activates the PI3K/AKT
pathway to promote inflammation of liver tissue (Wu et al., 2018). A study (Simonetti et al., 2006) manifested that CCL27-CCR10 can facilitate melanoma to disseminate to lymph nodes and escape the immune response. The results of this investigation demonstrated that the ligand (CCL27) of CCR10 exacerbated the malignant progression of LUSC. CCL27, a key chemokine in vivo, participates in multiple immune responses, inflammation occurrence, and immune escape (Homey et al., 2002; Simonetti et al., 2006). Besides, it is most involved in sensitization dermatitis and highly expressed in the serum of patients with psoriasis and sensitization dermatitis (Kakinuma et al., 2003). CCL27 is also involved in immune escape of cancer and restrains immune response, thereby driving cancer progression (Murakami et al., 2003). Researchers discovered that CCL27 plays an oncogenic role in cancers such as melanoma (Simonetti et al., 2006; Monteagudo et al., 2012). We performed cell experiments and revealed that CCL27-CCR10 markedly enhanced cell proliferative, migratory, and invasive capabilities of LUSC cells. This promoting effect can be weakened by the simultaneous CCL27 treatment and CCR10 knockdown. Together with these results, CCL27-CCR10 markedly facilitated progression of LUSC.

Both key chemokines and the downstream pathways of chemokines attract researchers’ interest. Several investigations reported that chemokine signaling pathways affect cancer progression via relatively downstream pathways. For instance, RDM1 stimulates HCC occurrence and progression via Ras/Raf/ERK pathways (Chen et al., 2020). TXNDC12 facilitates HCC progression by promoting epithelial-mesenchymal transition (EMT) (Yuan et al., 2020). EMT, ERK, Warburg, JAK, NF-kB, and some other pathways are mainly involved in the occurrence and progress of cancers (An et al., 2021; Gao et al., 2021; Jain et al., 2021; Kaplan et al., 2021). PI3K/AKT is well understood as an important oncogenic pathway that positively activates tumor-promoting cell behaviors, such as cell survival, apoptosis, and cancer drug resistance (Nishida, 2021). In this study, we speculated that CCL27-CCR10 may lead to PI3K/AKT pathway activation and carried out western blot for confirmation. The result presented that CCL27-CCR10 was able to activate the PI3K/AKT pathway. In addition, AKT inhibitor A6730 blocked CCL27-mediated PI3K/AKT pathway
activation, as well as the promoting effect of CCL27 on cell proliferation, migration, and invasion in LUSC. PI3K/AKT is a considerable oncogenic pathway that facilitates tumor growth via varying downstream pathways. It modulates the growth, metabolism, autophagy, and apoptosis of cancer cells via the mTOR pathway (Wang et al., 2018; Yu et al., 2019). The PI3K/AKT/p27 axis also regulates the cancer cell cycle (Zhou et al., 2018; Nam et al., 2019). Thus, we concluded that the CCL27-CCR10 regulatory axis stimulated the malignant events of LUSC cells by activating the PI3K/AKT pathway.

Viewed in total, we confirmed through cell experiments that CCL27-CCR10 promoted cell proliferation, migration, and invasion in LUSC. Additionally, CCL27-CCR10 activated PI3K/AKT to facilitate disease occurrence and progression. One major drawback of this study is that we only conducted cellular molecular experiments and certain bioinformatics explorations despite rigorous experiments and reliable conclusions. Further exploration of animal models is therefore warranted. Rigorous animal experiments and clinical trials will be incorporated into the plan for further research.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Competing interests**

The authors declare that they have no potential conflicts of interest.

**Funding**

This study was supported by the funds from National Natural Science Foundation of China (No. 82060078) and Natural Science Foundation of Guangxi Province (No. 2016GXNSFAA380196). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
Availability of data and material

The data used to support the findings of this study are included within the article. The data and materials in the current study are available from the corresponding author on reasonable request.

Authors' contributions

Dr. Baijun Li and Dr. Caizhou Wei contributed to the study conception and design. Material preparation, data collection and analysis were performed by Dr. Yonglong Zhong. The first draft of the manuscript was written by Dr. Baijun Li and Dr. Jianwei Huang. Dr. Caizhou Wei and Dr. Rizhu Li revised the manuscript. Dr. Rizhu Li gave the final approval of the version to be submitted. All authors read and approved the final manuscript.

References

Clin. Oncol. 34, 2980-2987.


via pi3k/akt pathway activation. Cell Death Dis. 9, 232.

Figure legends

Figure 1 CCR10 affects LUSC patients’ survival and is highly expressed in LUSC cells
A: Survival curves of high/low CCR10 expression groups; B: Relative CCR10 mRNA expression in normal lung cell line (HLF-a) and LUSC cell lines (SK-MES-1, NCI-H520, NCI-H1703, EBC-1) were evaluated by qRT-PCR; C: CCR10 protein expressions in normal bronchial epithelial cell line and LUSC cell lines were examined by western blot; * p<0.05.

Figure 2 CCL27-CCR10 hastens LUSC cell proliferation, migration, and invasion
A-B: CCR10 mRNA and protein expression levels in the differently-treated groups were measured by qRT-PCR and western blot respectively; C: CCK-8 assay was used to examine cell viabilities in the differently-treated groups; D: Colony formation assay was applied to evaluate colony formation ability in the differently-treated groups; E: Migratory and invasive abilities of the cells in the differently-treated groups were analyzed by Transwell assay; * p<0.05 and ns mean not significant.
Figure 3 CCL27-CCR10 can activate the PI3K-AKT pathway
The expression levels of CCR10 and the PI3K/AKT signaling pathway-related proteins were evaluated by western blot; * $p<0.05$ and ns mean not significant.

Figure 4 CCL27 facilitates the malignant progression of LUSC cells by activating the PI3K/AKT pathway
A: The expressions of CRC10 and the PI3K/AKT signaling pathway-related proteins were evaluated by western blot in the differently-treated groups; B-C: CCK-8 and colony formation assays were used to examine the cell viabilities in the differently-treated groups; D: Transwell assay was used to examine cell migratory and invasive abilities in the differently-treated groups; * $p<0.05$. 
**A**

Overall Survival

- **Low ccr10 Group**
- **High ccr10 Group**

Logrank $p=0.013$

HR(high) = 1.6

$p$(HR) = 0.014

$n$(high) = 120

$n$(low) = 110

**B**

Relative CCR10 mRNA expression

- HLF-a
- EBC-1
- NC-H1703
- SK-MES-1
- NCI-H520

* indicates significance.

**C**

Relative CCR10 protein expression

- HLF-a
- EBC-1
- NC-H1703
- SK-MES-1
- NCI-H520

* indicates significance.
HISTOLOGY AND HISTOPATHOLOGY

A = ..., ~

1.5 u ...
oeo oo 1.0 ~ Q.

CCL27
sh-CCR10
CCR10
\beta\text{-actin}

B

Relative mRNA expression of CCR10

<table>
<thead>
<tr>
<th>Condition</th>
<th>CCL27</th>
<th>sh-CCR10</th>
<th>CCL27</th>
<th>sh-CCR10</th>
</tr>
</thead>
<tbody>
<tr>
<td>sh-NC + PBS</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>sh-NC + CCL27</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>sh-CCR10 + PBS</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>sh-CCR10 + CCL27</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Relative protein expression of CCR10

C

Cell viability (%)

D

Migration

Invasion

sh-NC

sh-CCR10

CCL27

PBS

Number of colonies

E

Number of cells

Migration

Invasion

CCL27

sh-CCR10

sh-NC + PBS

sh-NC + CCL27

sh-CCR10 + PBS

sh-CCR10 + CCL27
CCL27

sh-CCR10

CCR10

PI3K

Akt

p-Akt

β-actin

Relative protein expression

<table>
<thead>
<tr>
<th></th>
<th>CCR10</th>
<th>PI3K</th>
<th>p-AKT/AKT</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>+</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>+</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* ns

*