The role of CXCR3 in renal cell carcinoma metastasis

（腎細胞癌の転移における CXCR3 の役割）

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Abstract

Background: Renal cell carcinoma (RCC) is known to express CXCR3. The function of CXCR3 on RCC has not been clarified. The aims of this study were to reveal the function of CXCR3 on RCC and to investigate the regulating factors of CXCR3.

Methods: Fifty-six clinical samples of clear cell RCC and corresponding normal renal tissue samples were obtained from surgical specimens of Japanese patients who underwent radical nephrectomy at Chiba University Hospital between 2000 and 2011. As RCC cell lines, 786-O, ACHN, and Caki-1 were used. The expression profile of CXCR3 and its splice variants were examined. For functional analyses, 786-O and IFN-γ-inducible 10-kDa protein or IP-10 (CXCL10) were selected as the representative.

Results: CXCR3 and its ligands were abundant in RCC samples compared with corresponding normal kidney samples. The CXCR3-A/CXCR3-B ratio was 1.5 times higher in RCC samples than in normal kidney samples. CXCL10 treatment induced 786-O cell migration and invasion; these effects were inhibited by neutralizing antibody. The expressions of phosphorylated RhoA and pro/active MMP-9 were up-regulated by CXCL10 treatment. In clinical samples, the expressions of CXCR3 and CXCR3-A were significantly higher in metastatic RCC than in non-metastatic RCC. Finally, the expression of CXCR3 and hypoxia-inducible factor 1 alpha (HIF-1-alpha) were
significantly correlated in the clinical samples. Regarding 786-O, the treatment with cobalt chloride (CoCl$_2$) up-regulated CXCR3 and HIF-1-alpha expression 4.5-fold and 2.2-fold, respectively.

Conclusions: CXCR3 plays important roles in RCC metastasis. The expression of CXCR3 may be regulated by hypoxia.
**Introduction**

Renal cell carcinoma (RCC) accounts for up to approximately 3% of new cancer cases and deaths in the Western world. At presentation, 20-30% of all RCC patients are diagnosed with metastatic disease [1]. In addition, 30-35% of RCC patients who undergo nephrectomy will experience relapse and develop metastatic RCC (mRCC) [2]. For patients with mRCC, the prognosis is extremely poor. Although many studies are being conducted with the aim of revealing the mechanisms of RCC metastasis, these mechanisms have not yet been well documented.

Chemokines are a superfamily of small (7-16 kDa), pro-inflammatory chemoattractant cytokines, which were originally characterized by their ability to induce migration of leukocytes [3, 4]. Recently, chemokines have also been demonstrated to play a major role in tumor metastasis [5-9]. Many kinds of cancers are found to express chemokine receptors, and their corresponding ligands are expressed by the sites of tumor metastases [5, 8, 10, 11].

Over 50 chemokines and 20 chemokine receptors have been identified so far. The chemokine receptors are divided into four subgroups (CXC, CC, CX3C, and C) depending on the position of the conserved cysteine residues from the amino terminal end of these proteins [12]. CXCR3 is one of the relatively well-documented subgroups,
a classic seven-transmembrane G-protein-coupled CXC chemokine receptor which has been reported to be expressed on activated T lymphocytes [13]. CXCR3 has been observed to induce calcium flux and chemotaxis in response to its ligands, monokine induced by human interferon-gamma (IFN-γ) or Mig (CXCL9), IFN-γ-inducible 10-kDa protein or IP-10 (CXCL10), and interferon-inducible T-cell alpha chemoattractant or I-TAC (CXCL11) [14].

In a previous study, we first reported that CXCR3 is expressed in RCC, and we indicated that CXCR3 should play an important role in tumor metastasis [15]. To date, a few reports have become available discussing the relationship between RCC and CXCR3 [16-18], but the function of CXCR3 in RCC metastasis has not yet been revealed. To clarify the function of CXCR3 in RCC metastasis, we evaluated the expression of CXCR3 in various RCC clinical samples and performed in vitro assays using human RCC cell lines.

Using clinical samples, we demonstrated the relationship between CXCR3 and RCC metastasis. We also revealed that the CXCR3/CXCL10 axis induced tumor migration and invasion. Furthermore, we investigated the mechanism of how the CXCR3/CXCL10 axis induced cell migration and invasion, and how the expression of CXCR3 is regulated.
Material and Methods

Clinical samples

Fifty-six clinical samples of clear cell RCC and corresponding normal renal tissue samples were obtained from surgical specimens of Japanese patients who underwent radical nephrectomy at Chiba University Hospital between 2000 and 2011. None of the patients had received adjuvant therapy before surgery. Written informed consent was preoperatively obtained from each patient. Parts of the tissues were frozen immediately with liquid nitrogen and were kept at -80°C until analysis. The remaining tissues were fixed with 10% formalin and embedded in paraffin. Hematoxylin and eosin staining was also performed. The clinicopathological features of the patients with clear cell RCC are detailed in Table 1.

Cell lines, cell culture, and treatment with cobalt chloride

The cell line 786-O was from American Type Culture Collection (ATCC® Manassas VA, USA), and the cell lines ACHN and Caki-1 were supplied from The Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Miyagi, Japan). Authentication of cell lines was achieved by Short Tandem Repeat profiling. These cell lines were passaged in our laboratory for fewer than 6 months after resuscitation. These cell lines were cultured in RPMI-1640 medium
supplemented with 10% fetal bovine serum (FBS), and were maintained in an incubator with a humidified atmosphere of 95% air and 5% CO₂ at 37°C. To induce chemical hypoxia, 500 μM of cobalt chloride (CoCl₂) was added to the medium and the cells were treated for 24 hours.

**Real-time reverse-transcription polymerase chain reaction (RT-PCR) analysis**

Total RNA was extracted from the frozen surgical specimens and cell lines using the RNeasy Mini Kit (QIAGEN, Venlo, Netherlands) according to the manufacturer’s instructions. Complementary DNA was synthesized using the ImProm-II™ Reverse Transcription System with random primer (Promega, Tokyo, Japan). Real-time RT-PCR was performed using an ABI7300 Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA). The PCR reactions were performed in a final volume of 25 μL of a reaction mixture of SYBR® Green PCR Master Mix (Applied Biosystems). Conditions for real-time RT-PCR were as follows: at 50°C for 2 minutes, at 95°C for 10 minutes, 40 cycles at 95°C for 15 seconds, and at 60°C for 1 minute. The mRNA levels of tested genes were normalized to β-actin levels using a ΔCt method. In clinical samples, the mean expression ratios for tumor specimens were normalized with respect to the mean expression ratios for normal renal tissues. The primers for human CXCR3 were 5’-AGCTCTGAGGACTGCACCAT-3’ and 5’-CAGTCACTGCTGAGCTGCAA-3’; for
human CXCR3A, 5’-CCATGGTCTTGAGGTGAGT-3’ and 5’-CAGCAGAAAGAGGAGGCTGT-3’; for human CXCR3B, 5’-TGCCAGGCCTTTACACAGC-3’ and 5’-TCGGCGTCATTTAGCACTTG-3’; for human CXCL9, 5’-CCACCGAGATCCTTATCGAA-3’ and 5’-CTAACCGACTTGCTGCTTC-3’; for human CXCL10, 5’-GCAGAGGAACCTCCAGTCTCA-3’ and 5’-GCAGGTACAGCGTACGGTTC-3’; for human CXCL11, 5’-GGTGGGTAAAGGACCAAAA-3’ and 5’-CCGATGGTAACCAGCCTTTC-3’; for human hypoxia-inducible factor 1-alpha (HIF-1α), 5’-CCCAATGGATGATGACTTCC-3’ and 5’-TGGGTAGGAGATGGAGATGC-3’ and for human β-actin, 5’-CTCCTCCTGAGCGAAGTACTC-3’ and 5’-TCCTGCTTGCTGATCCACATC-3’.

Reagents and antibodies

Recombinant CXCL10 was purchased from R&D Systems (Minneapolis, MN, USA). Anti-CXCR3 and anti-pro/active matrix metalloproteinase-9 (MMP9) monoclonal antibodies (mAbs) were obtained from R&D Systems. Anti-Rho A and anti-phospho-Rho A (pSer188) were obtained from ABGENT (San Diego, CA, USA) and ECM Biosciences (Versailles, KY, USA).

Western blot analysis
Cells incubated on dishes were lysed with radioimmunoprecipitation assay (RIPA) buffer (150 mmol/L NaCl, 50 mmol/L Tris (pH 8.0), 0.5% deoxycholic acid, 1% NP-40 and 1 mmol/L phenylmethylsulfonyl fluoride). Protein samples (30 μg) were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to Hybond-C membranes (GE Healthcare, Little Chalfont, UK). The membranes were blocked in 5% milk TBS-T (tris-buffered saline (TBS) containing 0.05% Tween® 20; 1 hour at room temperature), and, following incubation with the respective primary antibody (1:100 dilution, overnight at 4°C), membranes were exposed to species-specific horseradish peroxidase-labeled secondary antibodies. Signals were detected using the ECL Plus Western Blotting Detection System (GE Healthcare) and visualized using LAS-4000 mini software (Fujifilm, Tokyo, Japan).

**Immunohistochemistry**

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded sections using anti-CXCR3 mAbs. Briefly, after deparaffinization and hydration, the slides were treated with endogenous peroxidase in 0.3% hydrogen peroxide solution in 100% methanol for 30 minutes, after which the sections were blocked for 2 hours at room temperature with 1.5% blocking serum in PBS before reacting with anti-CXCR3 mAbs (1:100 dilution) at 4°C in a moist chamber overnight. Following incubation with the
primary antibody, the specimens were washed three times in PBS and were then treated with the EnVision™ (Dako, Glostrup, Denmark) reagent followed by color development in 3,3’-diaminobenzidine tetrahydrochloride (Dako, Carpinteria, CA). Finally, the slides were lightly counterstained with hematoxylin, dehydrated with ethanol, cleaned with xylene, and mounted.

**Wound healing assay**

Cultured monolayers of 786-O cells were wounded with a pipette tip and cultured for a further 16 hours in starvation medium with a different concentration of CXCL10. PBS was used as negative control. The cells were visualized by light microscopy at 0 and 16 hours after scratch. The migration rate was calculated using Lenaraf220b software (Atelier M&M, Japan) by measuring the distance traveled by the cells, using the following formula: migration rate = distance from the edge (0 hour) – the distance from the edge (16 hours)/ the distance from the edge (0 hour). All experiments were repeated four times.

**Migration assay**

Migration was assayed in 24-well Transwell cell culture chambers (8-µm pores; Coster, Cambridge, MA, USA). After 786-O cells were added to the upper chamber (5×10^4 cells/well) and incubated for 8 hours, cells attached on the lower surface of the
membrane were counted in at least five fields (original magnification, 200×). PBS was used as negative control. At least three experiments were performed for each set. For neutralization studied, cells were incubated with anti-CXCR3 mAb in the upper chamber.

**Invasion assay**

Invasion was assayed in 24-well BD BioCoat\textsuperscript{TM} Matrigel\textsuperscript{TM} Invasion Chambers (BD Biosciences, Franklin Lakes, NJ, USA). After 786-O cells were added to the upper chamber ($5\times10^4$ cells/well) and incubated for 16 hours, cells attached on the lower surface of the membrane were counted in at least five fields (original magnification, 200×). PBS was used as negative control. At least three experiments were performed for each set. For neutralization studied, cells were incubated with anti-CXCR3 mAb in the upper chamber.

**Statistical analysis**

Values were expressed as the mean ± standard error. The Mann-Whitney U test and the Wilcoxon signed-rank test were used for statistical evaluation of real-time PCR data. Statistical significance was defined as $P < 0.05$. All statistical analyses were performed using SAS version 9.2 software (SAS Institute, Cary, NC, USA).
Results

**CXCR3 and its splicing variant expression on clinical samples and on RCC cell lines**

In our previous study, we revealed that RCC expressed CXCR3. In the present study, we examined the expression of CXCR3 and its splicing variants CXCR3-A and CXCR3-B in clinical samples by real time RT-PCR. The expression of CXCL9, CXCL10 and CXCL11 were also examined. The patients’ characteristics are listed in Table 1. All patients had been diagnosed with clear cell RCC. At the time of the surgery, 15 patients had metastases and 41 patients had no metastasis. We confirmed that the expression of CXCR3 and its ligands are highly up-regulated in renal cancer tissues compared with adjacent normal kidney tissues (Fig. 1A). The expression levels of CXCR3, CXCL9, CXCL10 and CXCL11 in RCC tissues were about 6 times greater, 12 times greater, 22 times greater, and 8 times greater, respectively, compared with the levels in the corresponding normal kidney tissues.

The expression ratio of CXCR3-A/CXCR3-B was also calculated. The average CXCR3-A/CXCR3-B ratio was 1.48 in renal cancer tissues and 1.01 in normal kidney tissues. In cancer tissues, the dominance of CXCR3-A expression was demonstrated by immunohistochemistry; we confirmed that CXCR3 expression was coincident with the
RCC cell membrane (Fig. 1B). The antibody used for immunohistochemistry could not distinguish between CXCR3-A and CXCR3-B. According to the real-time RT-PCR results, more than three-fifths of the staining could be considered as CXCR3-A.

As the next step, we examined the expression of CXCR3 on RCC cell lines. By real-time RT-PCR, we confirmed that RCC cell lines expressed CXCR3, and the highest expression was observed on the 786-O cell line (Fig. 1C). Then, we investigated the protein expression level of CXCR3 (Fig. 1D) by Western blot analysis. At the protein level, the 786-O cell line was proved to express CXCR3 at the highest level among the three study cell lines (Fig. 1D). Moreover, the CXCR3-A/CXCR3-B expression ratio was equivalent in the 786-O cell line and in the clinical samples. The CXCR3-A/CXCR3-B ratio on the 786-O cell line was 1.7. For these reasons, we selected the 786-O cell line for further analysis. We next examined whether 786-O secreted CXCL9, CXCL10 and/or CXCL11. Examination by enzyme-linked immunosorbent assay (ELISA) using 786-O culturing supernatant revealed that 786-O did not secrete any of the three ligands CXCL9, CXCL10 or CXCL11 (data not shown).

**CXCL10 induced RCC cell line migration and invasion**

CXCR3 has three ligands: CXCL9, CXCL10, and CXCL11. It is thought that there is no functional difference between these three ligands. In clinical samples, CXCL10 was
the highest expressed ligand among these three. We therefore selected CXCL10 as the representative ligand for functional analysis.

For functional analysis of the CXCR3/CXCL10 axis, we first examined the proliferation of 786-O treated with CXCL10. The cell line 786-O was cultured with or without various concentrations of CXCL10, and CXCL10 did not alter cell proliferation (data not shown). Then, we conducted migration analyses using a wound healing assay and a migration assay kit. Migration of 786-O increased 1.8-fold with CXCL10 treatment, in comparison with control. Migration change was observed in a concentration-dependent manner, with the maximum effect observed in 100 ng/mL of CXCL10 (Fig. 2A). For further analysis, the concentration of CXCL10 was fixed at 100 ng/mL. Using a migration assay kit, a similar result was obtained (Fig. 2B); CXCL10 treatment up-regulated 786-O migration activity approximately 1.7-fold, and neutralizing the CXCR3 antibody inhibited its effect. Then, we examined whether CXCL10 treatment would influence the invasion of 786-O. Using an invasion assay kit, CXCL10 treatment up-regulated invasion activity approximately 1.8-fold; its effect was also inhibited by the neutralizing CXCR3 antibody (Fig. 2C).

Moreover, in analyses of clinical samples, the relative expression level of CXCR3 (tumor/normal) was as much as 3 times higher in the patients with metastases compared
with the patients without metastasis ($P < 0.05$) (Fig. 2D (1)). The CXCR3-A/CXCR3-B ratio in the metastasis group was also significantly higher compared with the non-metastasis group ($P < 0.05$) (Fig. 2D (2)).

**How migration and invasion were induced**

To investigate the mechanisms of how the CXCR3/CXCL10 axis promoted cell mobility, we first studied the expression change of phosphorylated RhoA. Adding CXCL10 increased the expression of phosphorylated RhoA. The expression increased from 5 minutes to 10 minutes after treatment; then it began to decrease gradually to 30 minutes after treatment (Fig. 3A).

Second, we examined pro/active MMP-9 induction by CXCL10 treatment. Under general conditions, 786-O does not secrete pro/active MMP-9. After treatment with CXCL10 for 24 hours, we detected pro/active MMP-9 expression in culturing supernatant by Western blotting. The strongest expression was observed at the 100-ng/mL concentration of CXCL10 (Fig. 3B).

**Hypoxia induced CXCR3 expression**

The expression level of CXCR3 and HIF-1-alpha was significantly correlated in clinical samples at the mRNA level (Fig. 4A). This result suggested that HIF-1-alpha might regulate CXCR3 expression.
To confirm this suggestion, we treated the three RCC cell lines (786-O, Cali-1, and ACHN) with cobalt chloride (CoCl₂), which mimics a low-oxygen condition. After CoCl₂ treatment, CXCR3 and HIF-1-alpha expression were induced only in the 786-O cell line; the expression of CXCR3 and HIF-1-alpha was up-regulated 4.5-fold and 2.2-fold after treatment (Fig. 4B). In Caki-1 and ACHN, the expression of CXCR3 and HIF-1-alpha was not induced. One of the fundamental differences between 786-O and the other two cell lines is in their type of von Hippel-Lindau (VHL) protein. The cell line 786-O has the mutated VHL protein; in contrast, Caki-1 and ACHN have the wild-type VHL protein. Wild-type VHL protein acts to prevent HIF-1-alpha accumulation; this is the reason why CoCl₂ induced CXCR3 and HIF-1-alpha expression only in the 786-O cell line.
Discussion

CXCR4 is known as one of the key players in RCC metastasis [16]. As for CXCR3, there have been several studies that examined CXCR3 and its relationship to RCC metastasis, and most of the reports considered CXCR3 as a favorable prognostic factor [17, 18]. In these reports, CXCR3 was considered to prevent cancer progression. On the other hand, in other types of the cancers, CXCR3 is considered as a poor prognostic factor, and one which promotes cancer metastasis [19-25]. CXCR3 is known to have at least two splice variants, CXCR3-A and CXCR3-B [26, 27]. In most of the previous reports discussing CXCR3 and cancer prognosis, the expression ratio of CXCR3-A/CXCR3-B was not mentioned. CXCR3-A has been shown to prompt cell proliferation and migration, whereas CXFR3-B has been shown to inhibit cell migration and induce apoptosis [26-28]. In *in vitro* analyses of RCC, CXCR3-B has been shown to work as a tumor suppressor [28-30]. The CXCR3-A/CXCR3-B ratio is important for determining tumor destiny.

Because reliable CXCR3-isoform specific antibodies are not available, we used real-time RT-PCR to quantify the expression level of these two splice variants. In the present study, we showed that CXCR3-A was more abundant than CXCR3-B in clinical renal cancer samples, and the same dominancy of CXCR3-A was observed in the 786-O
cell line. Under such conditions, we showed that CXCR3 expression was significantly related to metastasis in clinical samples, and the CXCR3/CXCL10 axis played an important role in tumor metastasis \textit{in vitro} using the 786-O cell line.

There have been a few reports which discuss the relationship between CXCR3 and hypoxia. Only in the breast cancer cell line, CXCR3 expression was reported to be regulated by CXCL10, which is induced by hypoxia [31]. As far as we know, the present study is the first report to reveal the relationship between CXCR3 and hypoxia both \textit{in vitro} and in clinical samples of RCC.

Furthermore, in our previous study, we revealed that tumor-associated macrophages (TAM) express CXCR3 [15]. TAMs are now well-recognized as promoting tumor progression [32]. TAMs are recruited to the ligands’ rich tumor sites, and help tumors to progress. These results shed new light on the mechanisms of how the microenvironment promotes tumor metastasis.

CXCR3 has the potential to be a candidate for a new therapy target because of its important roles in cancer progression. Although CXCR3 is reported to work as a regulator of inflammation, the immune reaction and angiogenesis [3, 4], the functions of CXCR3 are generally not necessary to maintain usual human activities. Moreover, it is easy to inhibit its functions by using the neutralizing CXCR3 antibody. CXCR3 is
considered to fulfill the requirements for the essential conditions as a molecular therapy target.

In three measured chemokines, CXCL10 has been observed to be the most abundant in clinical cancer tissues. Using ELISA, we examined whether we could measure CXCL10 expression in human serum. We could detect CXCL10 expression at a concentration similar to that used for experiments (50 ng/mL - 200 ng/mL) in clinical human serum. The expression level of CXCL10 was significantly correlated with the expression level of CXCR3-A (data not shown). CXCL10 is a potent tumor marker which predicts tumor metastasis and prognosis. Actually, in head and neck lymphoma, serum CXCL10 has well-reflected patients’ disease status [33]. As the next step, we are preparing to test serum CXCL10 expression as a tumor marker for predicting tumor prognosis.

In conclusion, the present study clarified the important role of the CXCR3/CXCL10 axis in tumor metastasis, and in CXCR3/CXCL10 interaction-induced cell migration and invasion. Hypoxia was indicated as regulative of CXCR3 expression. CXCR3/CXCL10 has the potential to be a target of new therapy. It is a candidate for becoming a novel biomarker that can be easily accessed in daily clinical situations.
References


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Table 1: Characteristics of the study subjects with clear cell renal cell carcinoma (RCC)

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FIGURE LEGENDS

Figure 1. Expression of CXCR3 and its ligands in clinical samples and RCC cell lines. A: The expression of CXCR3 and its ligands at the mRNA level in clinical samples. CXCR3 and its ligands are more abundant in tumor tissues (T) than corresponding normal tissues (N). *: \( P < 0.01 \). B: The localization of CXCR3 was examined by immunohistochemistry. (a) Hematoxylin and eosin (HE) staining of clear cell RCC (x200). (b) Immunohistochemistry of CXCR3 (x200). CXCR3 expression was coincident with cell membrane. C: CXCR3 mRNA expression in RCC cell lines. The expression of CXCR3 was approximately 3 times greater on 786-O compared with ACHN and Caki-1 at the mRNA level. D: CXCR3 protein expression in RCC cell lines. At the protein level, 786-O was proved to express CXCR3 at the highest level among these 3 cell lines.

Figure 2. Effect of CXCL10 on 786-O. A: Migration analysis using wound healing assay. (a) Pretreatment with phosphate buffered saline (PBS) (Control), (b) After 16 hours (Control), (c) Pretreatment with 100 ng/mL of CXCL10, (d) After 16 hours (100 ng/mL of CXCL10) The percentages of the scratched wounds’ fulfillment are presented as a bar graph. CXCL10 treatment induced cell migration in a concentration-dependent
manner. The influence reached a plateau at the 100 ng/mL concentration. B: Migration assay (original magnification, 200×). (a) Treatment with PBS, (b) Treatment with 100 ng/mL of CXCL10, (c) Treatment with PBS after neutralization, (d) Treatment with 100 ng/mL of CXCL10 after neutralization. The 100 ng/mL of CXCL10 induced 786-O cell migration, as we observed in the wound healing assay. This effect was inhibited by neutralizing antibody. The numbers of counted cells are displayed on the x-axis of the bar graph. C: Invasion assay (original magnification, 200×). (a) Treatment with PBS, (b) Treatment with 100 ng/mL of CXCL10, (c) Treatment with PBS after neutralization, (d) Treatment with 100 ng/mL of CXCL10 after neutralization. The 100 ng/mL of CXCL10 induced 786-O cell invasion by the same degree as migration. This effect was also inhibited by neutralizing antibody. D (1): Relative expression of CXCR3 in clinical samples. Expression of CXCR3 was significantly higher in patients with metastasis (M1) than without metastasis (M0) (*: P < 0.05). D (2): The expression ratio of CXCR3-A/CXCR3-B was also higher in M1 patients than M0 patients (*: P < 0.05).

Figure 3. **Mechanisms of induction of cell mobility.** A: Total amount of RhoA and the phosphorylation status of RhoA (Ser-188) were measured by Western blot analysis after the treatment with 100 ng/mL CXCL10. The expression of phosphorylated RhoA
(P-RhoA) increased from 5 to 10 minutes after treatment, then it began to decrease gradually by 30 minutes. The expression of the total amount of RhoA did not change. B: Pro/active MMP-9 was measured by Western blot analysis after 48 hours treatment. Pro/active MMP-9 was secreted in culturing supernatant. The strongest expression was observed at the 100ng/mL concentration of CXCL10.

**Figure 4.** A: The relationship between CXCR3 and HIF-1-alpha expression in clinical samples. The expression of CXCR3 and HIF-1-alpha was significantly correlated by real time RT-PCR. B: Expression changes of CXCR3 and HIF-1-alpha on 786-O cells after CoCl$_2$ treatment. CoCl$_2$ treatment, which mimicked hypoxia, induced CXCR3 and HIF-1-alpha expression on 786-O cells. The expression of CXCR3 and HIF-1-alpha was up-regulated 4.5-fold and 2.2-fold, respectively.
Figure 1

Figure 2
Figure 3

Figure 4
Cancer

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