Role of CXCL10 in angiogenesis of renal cell carcinoma

Hyun A Jin

Department of Medical Science

The Graduate School, Yonsei University

Role of CXCL10 in angiogenesis of renal cell carcinoma

Directed by Professor Sung Joon Hong

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Hyun A Jin

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of Hyun A Jin is approved.

Thesis Supervisor : Sung Joon Hong

Thesis Committee Member : Kyung Seok Han

Thesis Committee Member : Nam Hoon Cho

The Graduate School
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ABSTRACT

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Angiogenic process is complex and several factors are involved as a promoter or inhibitor in angiogenesis. Tumor can use the imbalance of pro- and antiangiogenic factors in progression. The CXC chemokine family is consisted of pro- or antiangiogenic factors. Recent studies revealed C-X-C motif chemokine 10 (CXCL10) as an anti-angiogenic factor in response to angiogenic factors like bFGF or VEGF. However, a role of CXCL10 in cancer and angiogenesis is unclear. Here, we investigated the role of CXCL10 on tumor angiogenesis and its mechanisms in renal cell carcinoma. CXCL10 inhibited proliferation and tube formation of HUVEC in a dose-dependent manner, while it did not affect in vitro tumor proliferation of renal cell carcinoma cells. Expression of CXCL10 was down-regulated in Caki-1, UMRC3 and UMRC6 cells. Overexpression of CXCL10 suppressed expression of angiogenic factors including HIF-1α, EGF, VEGFB, VEGFC, HGF, and MMP9 in Caki-1 cells. In vivo, restoration of CXCL10 expression suppresses tumor growth in renal cell carcinoma by suppressing tumor angiogenesis in xenografts. Our data suggest that targeting CXCL10 is a novel therapeutic option in renal cell carcinoma.

Key words : CXCL10, renal cell carcinoma, tumor angiogenesis

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I. INTRODUCTION

Angiogenesis is a pervasive biological event that is essential to several physiologic and pathologic processes, orchestrated by multiple stimulatory and inhibitory factors.[1,2] Angiogenesis is necessarily required for cancer growth and metastasis.[3] Recent advances in understanding of angiogenic mechanism have led the development of several anti-angiogenic agents which have proved survival benefits in several types of malignancies.[4-6] Therefore, anti-angiogenic therapies are expected to be effective in all the types of cancer but the therapeutic efficacy of these therapies is immensely variable.[7]

Renal cell carcinoma (RCC) is well known malignancy characterized by hypervascular tumor due to von Hip-pel-lindau (VHL) gene mutation.[8] VHL inactivation in most clear cell RCC tumors leads to VEGF and PDGF overexpression. VEGF is known to be a main angiogenic factor in tumor angiogenesis.[3-6] VEGF-targeting anti-angiogenic therapies have showed survival benefit in patients with renal cell carcinoma (RCC) and have become a standard first-line treatment in the management of RCC.[9] VEGF has been a main target in the current strategy of anti-angiogenic therapies. However, most of the tumors eventually become resistant to these agents and durable response is rare.[7] Various mechanisms have been suggested to explain resistance to anti-angiogenic therapies. One of the known mechanisms is that VEGF

targeting therapies upregulate several alternative angiogenic factors such as FGF-1-2, Ang-1 and EFNA1-2 as compensatory mechanisms to bypass blocked VEGF pathway.[7] These results suggest that blocking a single angiogenic factor is not sufficient to suppress tumor angiogenesis.

Various tumors induce imbalance of promoters and inhibitors of angiogenesis by enhancing pro-angiogenic factors and suppress anti-angiogenic factors to promote tumor growth and metastasis.[10] Therefore, restoration of the balance between pro-angiogenic factors and anti-angiogenic factors could be a way to overcome limitation of the anti-angiogenic therapy.

Chemokines are multifunctional mediators that can promote immune responses, stem-cell survival, development and homeostasis, as well as triggering chemotaxis and angiogenesis. Chemokines are divided into four subfamilies, based on structural properties and primary amino acid sequence: CXC, CC, C or CX3C.[2,10-13] Recent evidence indicates that CXC chemokine family have important role in the control of angiogenesis and has dual effects on angiogenesis, consisted of pro- or anti-angiogenic factors.[14,15]

Recently, C-X-C motif chemokine 10 (CXCL10), one of CXC chemokine family, has been investigated and several studies revealed that CXCL10 is an anti-angiogenic chemokine and is associated with anti-tumor activities.[16-19] CXCL10 was reported to antagonize the action of fibroblast growth factor (bFGF), thus suppressing angiogenesis induced by bFGF in advanced uterine endometrial cancers.[17] In estrogen receptor positive (ER+) mammary tumor, CXCL10 inhibits VEGF levels to reduce tumor burden.[18] Depletion of CXCL10 in squamous cell carcinoma (SCCA) tumors resulted in an increase in their size.

CXCL10 generally has a role negative regulator of tumor angiogenesis in other cancers, while the precise function and mechanisms of CXCL10 in RCC remains unclear. Therefore, we investigated roles and mechanisms of CXCL10 on tumor angiogenesis in RCC. Here, we showed that CXCL10 is downregulated in RCC cells and restoration of CXCL10 expression suppresses tumor growth in renal cell carcinoma by suppressing tumor angiogenesis in xenografts. Our data suggest that targeting CXCL10 is a novel therapeutic

option in renal cell carcinoma.

II. MATERIALS AND METHODS

1. Cell culture

Human clear cell type renal-cell carcinoma cell lines, Caki-1, Caki-2 and 786-O were maintained RPMI medium (Hyclone, Logan, Utah, USA) containing 10% fetal bovine serum (FBS; Hyclone). The other human renal-cell carcimona cell lines, UMRC-3 and UMRC-6 were maintained in MEM medium (Hyclone) containing L-glutamine and 10% fetal bovine serum (FBS; Hyclone). Human renal proximal tubular epithelial cell line (HK-2) were cultured in keratinocyte-serum-free medium (Gibco, Grand Island, NY, USA) containing 0.05mg/ml bovine pituitary extract (BPE), 5ng/ml human recombinant epidermal growth factor (EGF), 10% fetal bovine serum (FBS; Hyclone). Human umbilical vein endothelial cells (HUVEC) were maintained Medium 199/EBSS (Hyclone) containing 1μg/μl β-FGF, 1unit/ml heparin and 10% fetal bovine serum (FBS; Hyclone). Sterilized 2% gelatin (Sigma-aldrich, Louis, MO, USA) solution is used to culture plate for HUVEC cell plating. All cells were cultured at 37℃ in a humid atmosphere with 5% CO_2.

2. Tube formation assay

Low-passage HUVECs were cultured in Medium 199/EBSS with 10% fetal bovine serum (FBS; Hyclone) and were starved for 4hr before the tube formation assay. HUVECs were mixed with medium containing 1% fetal bovine serum (FBS; Hyclone) and then seeded on matrigel-coated 24-well plates at a density of 10^4 cells per well. Various doses (0, 1, 5, 10μmol/L) of CXCL10 (ebioscience, San diego, CA, USA) were added to the medium for tube formation assays. Tube formation was evaluated using a microscope and photographs were taken 3-18hr after seeding. The area covered by the tube network was determined with an optical imaging technique: pictures of the tubes were scanned into Adobe Photoshop and quantified using Image J software.

3. Cell viability assay

Cell viability was confirmed by 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxyme thonyphenol)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. Cells were prepared in 96-well plate containing 2.0 X 10^3 cells/well. Various doses (0, 1, 5, 10μmol/L) of CXCL10 (ebioscience) were added to the media. After 48hr of treatment, 20μl MTS solution were added to each well, and then incubated 3hr at 37℃. Absorbance was measured at 490nm using a spectrophotometer. All experiments were performed in triplicate and repeated a minimum of three times.

4. Cell cycle analysis

At 30-40% confluency, cells were synchronized at G0/G1 phase by eliminating serum from the culture medium for 24hr. After release from the G1 block by substitution with fresh media containing 10% fetal bovine serum (FBS; Hyclone), cells were maintained for 24hr. Cells were fixed with 70% ethanol at -20℃ overnight and then treated with 10μg/ml RNase and stained with 100μg/ml propidium iodide. Cell cycle phase was determined by flow cytometry. All experiments were performed in triplicated and repeated three times.

5. Cell proliferation assay

Cells were seeded in 96-well plates at a density of 5,000 cells/well, and cell proliferation was determined in triplicate using a Cell counting kit-8 reagent (CCK-8; Enzo life science, farmingdale, NY, USA) according to the supplier's protocols. At the indicated time points, cell number was measured spectrophotometrically as OD450 values of reduced WST-8 [2-(2-methoxy-4-ni trophenyl)-3-(4-nitrophenyl)-5-(2,4 disulfophenyl)-2H-tetrazolium, monosodiu m salt].

6. Western Blot analysis

For the detection of CXCL10, Cells were lysed in PRO-PREP protein extract solution (Intron; SungNam-si, Gyeonggi-do, Korea), preparing supernatant from cell culture media for western blot. The protein samples were separated on 12% SDS-TAGE and transferred to polyvinylidene fluoride membranes (Millipore; Darmstdt, Germany). The blots were incubated with primary antibodies (Santa cruz; Dallas, Texas, USA) and then with anti-mouse secondary antibodies conjugated to IgG -horseradish peroxidase (Santa cruz) in Tris-buffered Saline and Tween 20 solution (TBST; 1:1,000). Proteins were detected using the ECL western blotting substrate (Bionote; Hwaseong-si, Gyeonggi-do, Korea).

7. Establishment of stable CXCL10 overexpression cells

Lentiviral supernatants were produced by transfection of HEK293 cells using Trans-Lentiviral pachaging kits (Thermo; Walthan, MA, USA) according to the manufacturer's protocol. After 48hr, the tissue culture medium was filtered through a 0.45μm filter, and the viral supernatant was used for infection of Caki-1 cells. Caki-1 cells were infected for at least 24hr and allowed to recover for 48hr with fresh medium. Infected cells were selected with 10μg/ml Blasticidin.S (Sigma-aldrich) for 48hr.

8. Quantitative RT-PCR

Total RNA was isolated from cells with Trizol reagent (Invitrogen; Carisbad, CA, USA) according to the manufacturer's protocol. RNA was quantified by the ratio of absorbance at 260/280nm. First strand cDNA was synthesized from 0.5μg RNA by RT2 First Strand kit (Qiagen; Hilden, Germany), following the supplier's protocol. Human CXCL10 primer sequence is 5'- GAT GGA CCA CAC AGA GGC TG-3'(Forward), 5'-GAT GGG AAA GGT GAG GGA AA-3' (Reverse). GAPDH primer sequence is 5′-GGTGAAGGTCGGAGTCAACG-3′ and 5′-CACCATTCTCGCTCCTGGAAGATGGTG-3′. Quantitiative RT-PCR

for angiogenic factors was performed using RT2 profiler PCR Arrays (Qiagen) in combination with RT2 SYBR Green Mastermixes (Qiagen). All experiment was performed in triplicate and repeated a miniumum of three times.

9. Tumor xenograft models

BALB/c NUDE were purchased from Orient bio Inc. (SungNam-si, Gyeonggi-do, Korea) and maintained in accordance with the institutional guidelines of Yonsei University College of Medicine. A 6.0 x 10^6 Caki-1 cells in 100μl phosphate buffered saline (PBS) were injected subcutaneously into the flanks of 6-week-old female mice. After 7 days, Tumor volume was monitored every 4 days and measured using calipers. Tumor volume was calculated from a formula (tumor volume (mm^3) = width x length x height x 0.5). Mice were sacrificed after 49 days. For hypoxia staining, pimonidazole was injected intravenously 1hr prior to euthanasia. Mice were sacrificed and tumor tissues were extracted immediately so that the tissue hypoxia status was not affected.

10. Immunohistochemistry

Tumor were captured, and then were embedded in OCT compound and sectioned at 30μm thickness. Primary antibodies used were against CD31 (Abcam 1:50; Cambridge, United Kingdom) and pimonidazole (Hpi 1:100, Burlington, MA, USA). The sections were then incubated in Alexa Fluor-conjugated secondary antibodies (Life technology; Eugene, OR, USA). Sections were then photographed with a confocal microscope (Carl Zeiss LSM 700). Quantified analysis was used image J software.

III. RESULTS

1. In vitro extrinsic CXCL10 does not directly inhibit tumor proliferation in human renal-cell carcinoma cells, but effectively suppressed angiogenesis

We investigated the direct effects of the CXCL10 on cell proliferation in both renal-cell carcinoma and endothelial cells using cell viability assays. MTS assay after CXCL10 treatment demonstrated that tumor cell growth was not inhibited by CXCL10 in 786-0 cell, renal-cell carcinoma cell in a dose-dependent manner (Figure 1A). We also examined the effect of CXCL10 on cell cycle progression in CXCL10 knockdown 786-O cell using FACS analysis with propidium iodide staining. Cell cycle analysis showed that CXCL10 had no effect on cell cycle progression. (Figure 1B) In contrast, Tube formation assays with HUVEC revealed that proliferation was effectively inhibited by CXCL10 in dose-dependent manner (Figure 1C), indicating that extrinsic CXCL10 inhibited endothelial cell growth and angiogenesis, but not renal-cell carcinoma cell proliferation.

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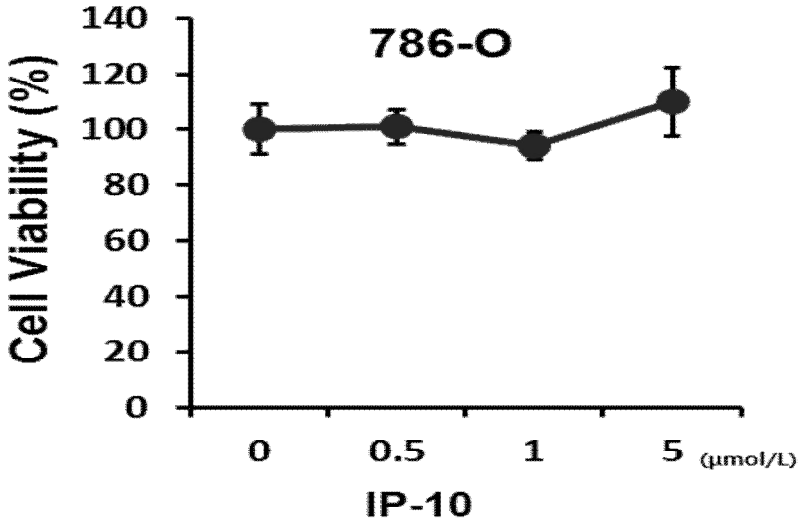

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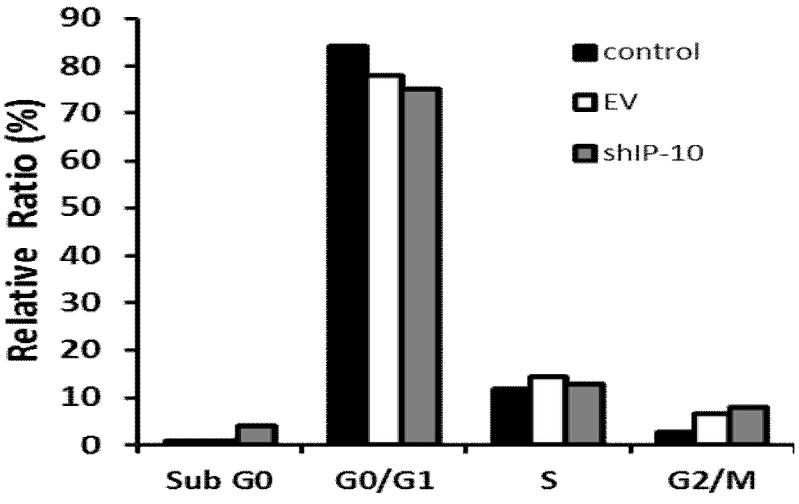

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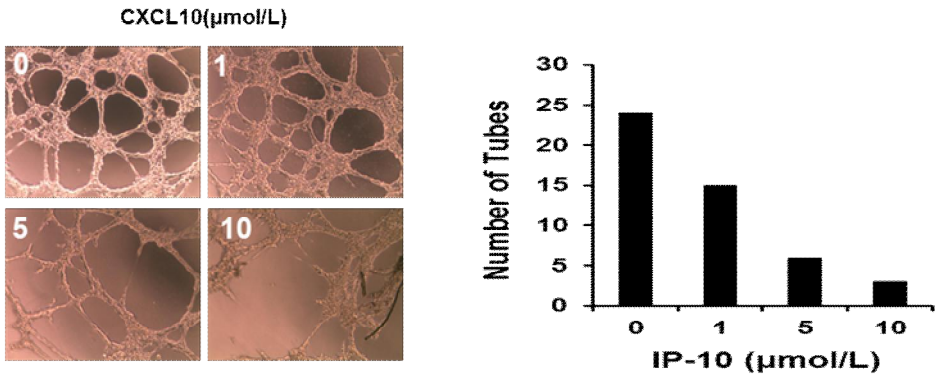

Figure 1. ***In vitro*** **effects of CXCL10 on cell proliferation in renal-cell carcinoma and endothelial cells.** Extrinsic CXCL10 inhibited proliferation and tube formation of vascular endothelial cell in a dose-dependent manner, but did not affect renal cell carcinoma cells. (A) 786-O cells were treated with CXCL10 (0, 0.5, 1, 5μmol/L) and cell viability was evaluated by MTS assay. The cell viability was represented with the relative absorbance. (B) To evaluate the effect of CXCL10 on cell cycle regulation, FACS analysis was performed in CXCL10 knock down 786-O cell. The proportion of each cell cycle phase was quantified. Data are expressed as percent total cells. (C) HUVECs were treated with CXCL10 (0, 1, 5, 10μmol/L) and then, tube formation assays is performed. The representative image of HUVECs was taken by a light microscope.

2. In vitro baseline expression of CXCL10 is down-regulated in Renal-cell carcinoma

We investigated baseline expression of CXCL10 in renal-cell carcinoma including Caki-1, Caki-2, 786-O, UMRC-3, UMRC-6 using RT-PCR (Figure 2A) and Western blotting (Figure 2B). Normal renal epithelial cell, HK-2 exhibited relatively strong expression level. In contrast, Low CXCL10 expression was observed in Caki-1, UMRC-3, and UMRC-6 compared to the other cell lines.

In order to gain further insight into the function of CXCL10 in renal-cell carcinoma, we selected the kidney cancer-derived Caki-1 cell line, which lowly expresses CXCL10, and generated Caki-1-derived cell lines stably overexpressing CXCL10 for further study. Western blotting (Figure 2C) and RT-PCR analyses (Figure 2D, E) confirmed the significantly increased levels of CXCL10 expression compared to control cell lines.

And then, we evaluated the effect of CXCL10 overexpression on cell proliferation in caki-1 cell using cell proliferation assay (Figure 2F). This result showed that CXCL10 overexpression had no effect on cell proliferation.

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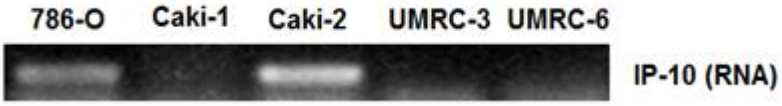

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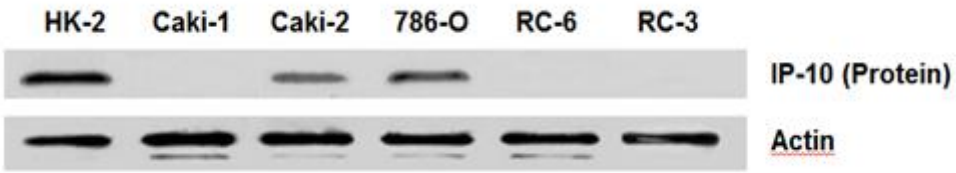

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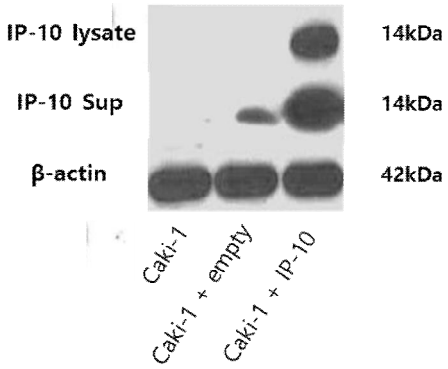

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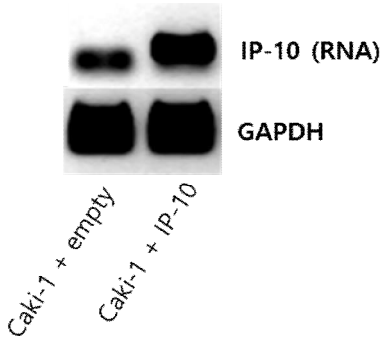

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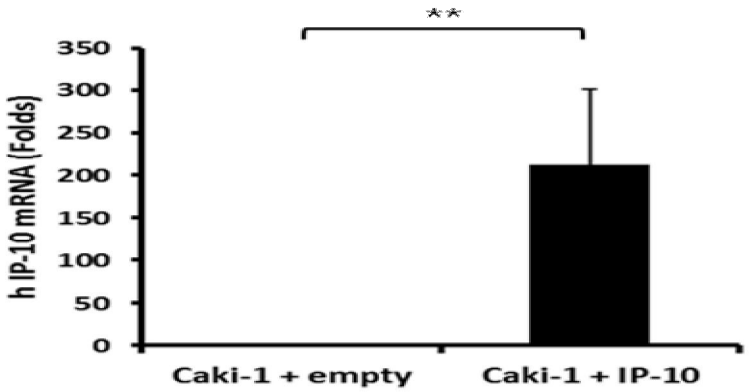

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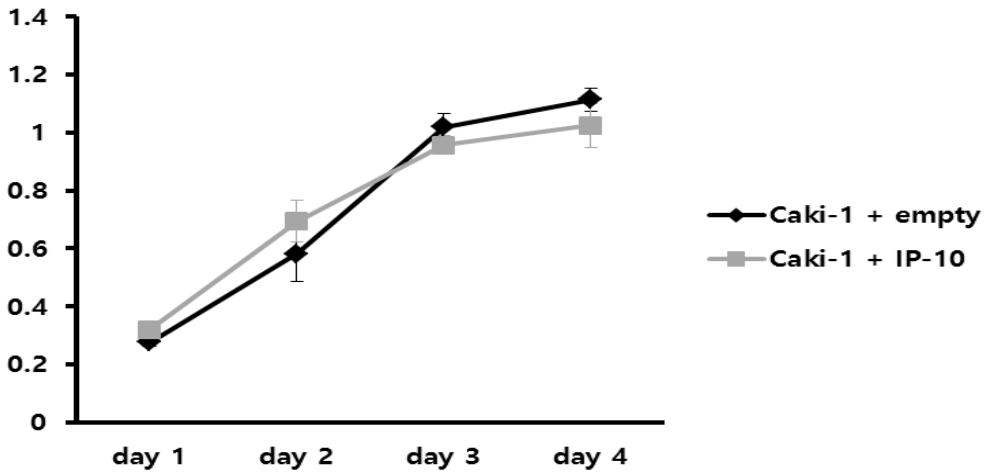

Figure 2. Baseline expression of CXCL10 in Renal-cell carcinoma and Generation of the CXCL10-overexpression Caki-1 cell line. Expression of CXCL10 was down-regulated in Caki-1, UMRC-3 and UMRC-6 cells. Assess the baseline expression of CXCL10 in a panel of renal cell carcinoma cells was confirmed by (A) RT-PCR and (B) Western blotting. Overexpression of CXCL10 was developed by lentiviral transfection. Overexpression validation was performed by (C) western blotting and (D, E) RT-PCR. The levels of GAPDH or β-actin were used as loading controls. Data represent the mean ± SD of three independent experiments. **P<0.001 (F) Cell proliferation assay was performed in CXCL10-overexpression Caki-1 cell line using CCK-8 reagent.

3. In vitro Overexpression of CXCL10 suppressed expression of angiogenic factors

The expression of angiogenesis related genes was assessed by real-time qRT-PCR using an optimized qPCR array. Expression of CXCL10 overexpression Caki-1 cells significantly down-regulated the expression of genes related to angiogenesis (MMP9, EGF, VEGFB, VEGFC, HGF, HIF-1α), as compared to controls (Figure 3A).

Among these down-regulated genes, Hypoxia-inducible factor-1α (HIF-1α) is known as a transcription factor, regulating the expression of angiogenic factor.[20] In addition, HIF-1α protein level also was down-regulated in CXCL10 overexpression cells (Figure 3B). These results suggest that CXCL10 is involved not only as an anti-angiogenic factor but also as a suppressor of critical angiogenic factors in renal cell carcinoma.

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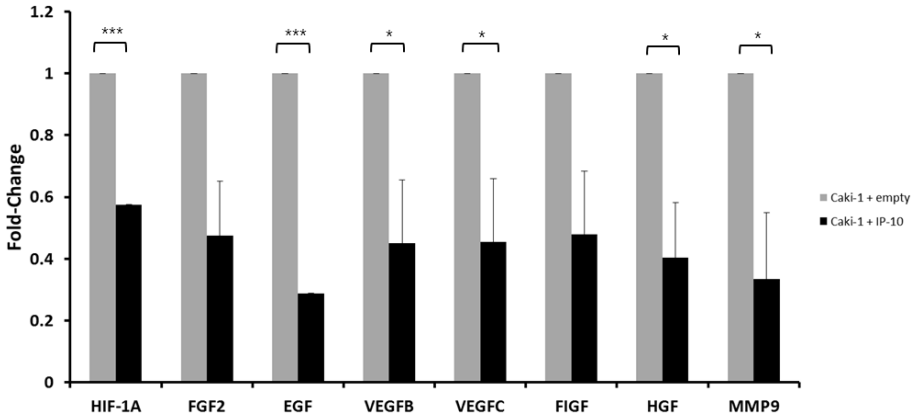

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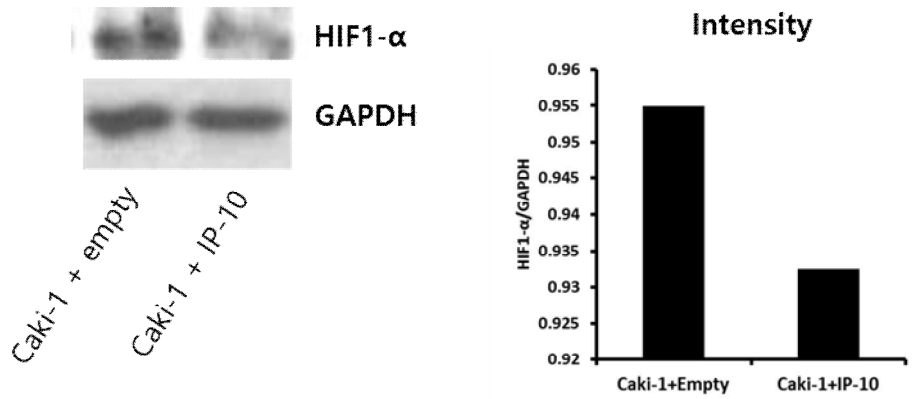

Figure 3. *In vitro* effects of CXCL10 overexpression on expression patterns of pro-angiogenic factors in Caki-1 cells. Overexpression of CXCL10 suppressed expression of pro-angiogenic factors including HIF-1α, EGF, VEGFB, VEGFC, HGF, and MMP9 in Caki-1 cells. (A) Quantitative RT-PCR array signatures of angiogenesis-related genes. Data represent the mean ± SD of three independent experiments. (B) Western blotting was performed for HIF-1α expression to evaluated effects of CXCL10. *$p<0.05$, ***$p<0.0001$

4. In vivo Restoration of CXCL10 expression suppress tumor growth in renal cell carcinoma

To investigate the effect of CXCL10 expression on tumor growth in vivo, BALC/c nude mice were inoculated subcutaneously with Caki-1+empty or Caki-1+IP-10 cells. The tumor grow rate in mice inoculated with Caki-1+IP-10 cells was significantly lower than control mice inoculated with Caki-1+empty cells. (Figure 4 A, B). The sizes of the dissected tumor tissues after sacrifice corroborated these findings (Figure 4C).

After sacrifice, the xenografts were immunohistochemically examined for CD31 to confirm tumor vasculature. In tumors from control mice, microvessel density was relatively high, whereas tumors from mice inoculated with CXCL10 restored Caki-1 cells shows reduced microvessel density (Figure 4D). These results suggested that consistent with the in vitro data, in vivo restoration of CXCL10 expression inhibits tumor growth in renal cell carcinoma by suppressing tumor angiogenesis in xenografts.

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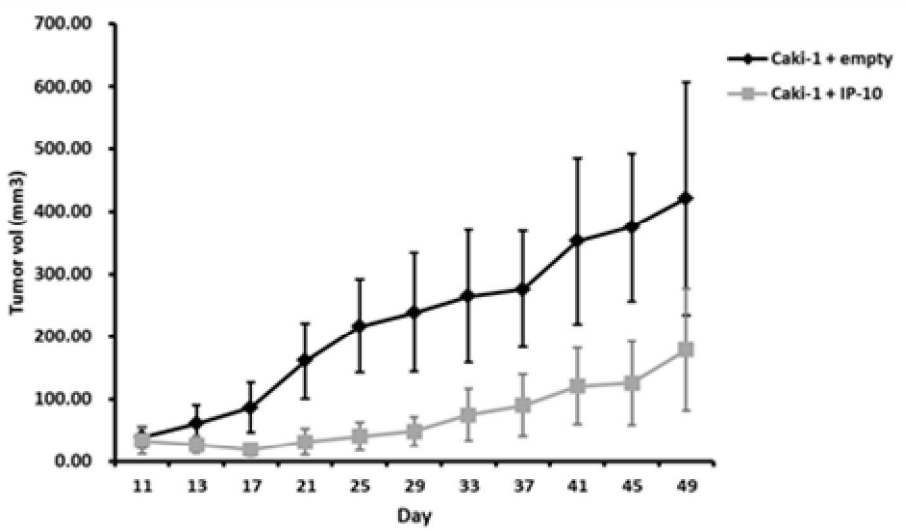

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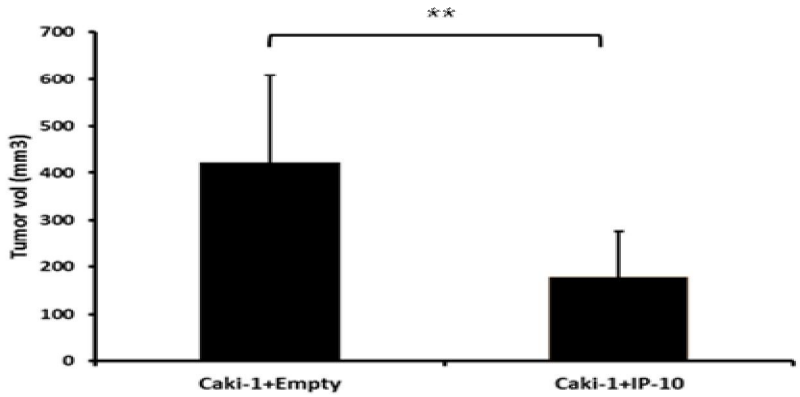

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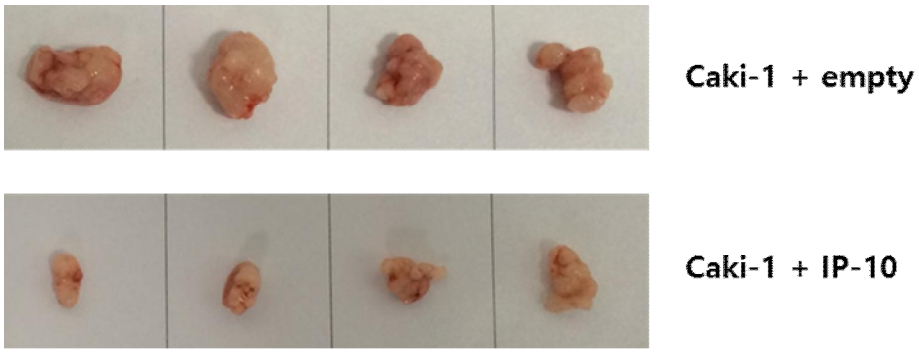

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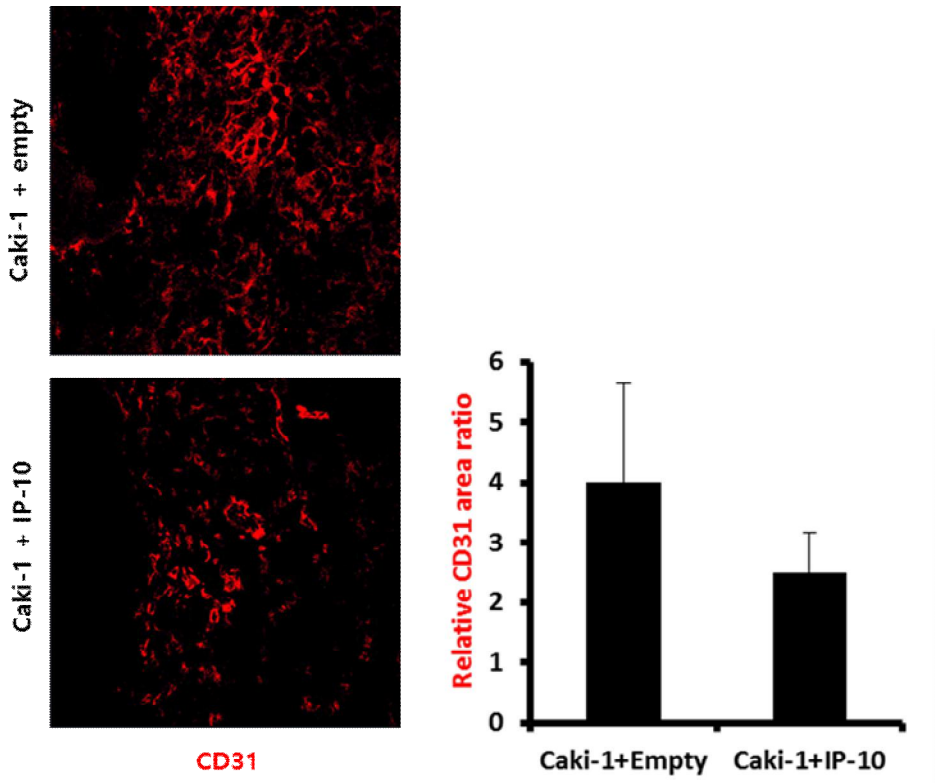
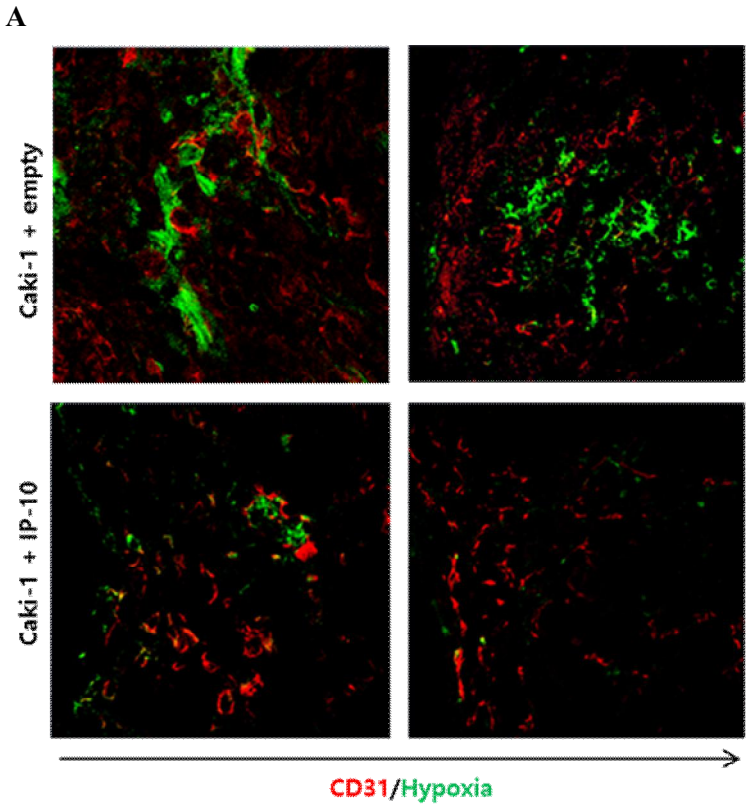


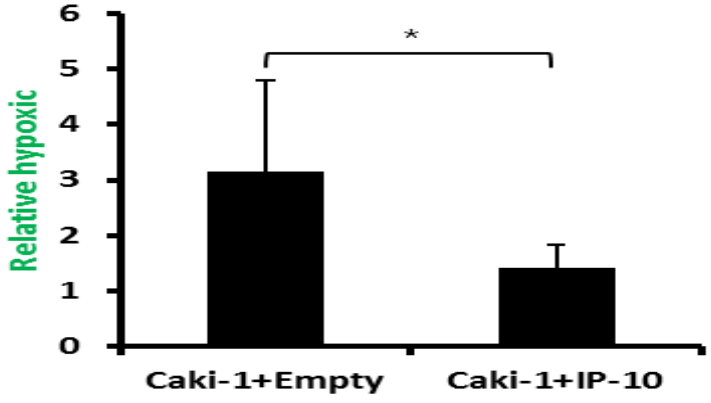
Figure 4. ***In vivo*** **effect of CXCL10 restoration in the development of tumor cell xenograft.** Caki-1-derived cell lines stably overexpressing CXCL10 were inoculated in to the flank of nude mice. Restoration of CXCL10 expression suppresses tumor growth in renal cell carcinoma. (A) Representative photograph of resected tumors from the two groups of mice at the time that they were sacrificed. (B) Tumor volume changes in the two groups of mice. After day 7, tumor volume was monitored every 4 days. (C) Relative tumor size at the end point of the in vivo study **P<0.001 (D) Tumor section were stained for CD31, a vascular epithelial cell marker, using immunofluorescence.

5. Restoration of CXCL10 expression suppresses tumor angiogenesis and reduces tumor hypoxia

We investigated the hypoxia condition in the tumor xenografts using immunofluorescence staining with an anti-pimonidazole antibody. Interestingly, we found that strong pimonidazole staining intensities were observed in mice inoculated with Caki-1+empty cells. In contrast, pimonidazole expression was relatively low in mice inoculated with Caki-1+IP-10 cells (Figure 5A).

To understand these results, hypoxia-inducible factor (HIF-1α) was stained using immunofluorescence. HIF-1α also was reduced in mice inoculated with Caki-1+IP-10 cells (Figure 5B). These results show that CXCL10 successfully induced limited hypoxia in tumor cells despite of inhibiting tumor angiogenesis in renal cell carcinoma.





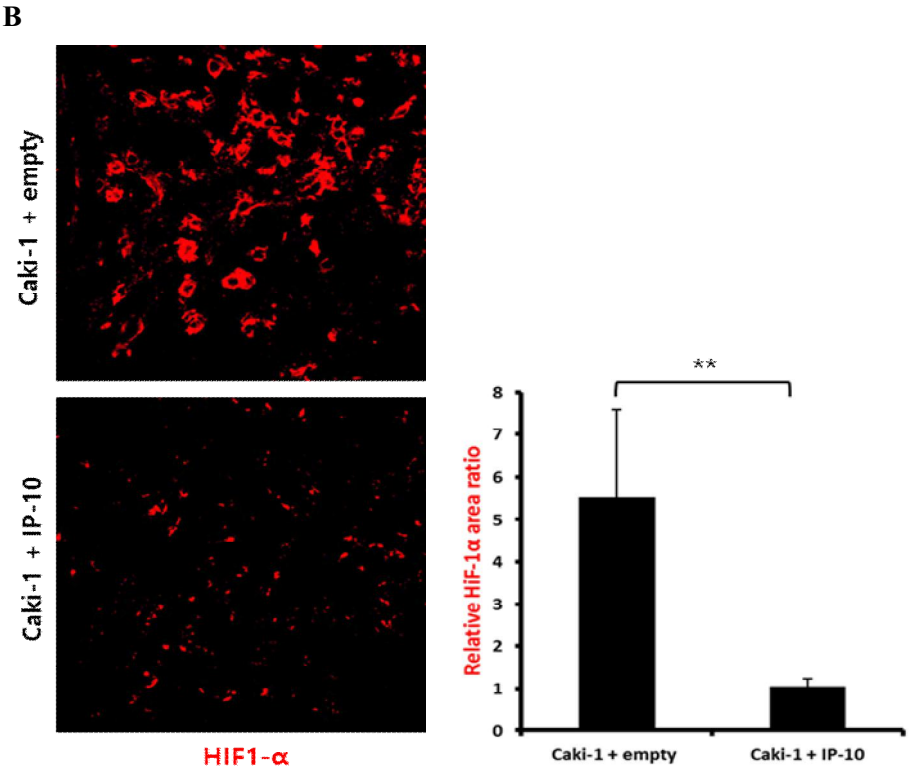


Figure 5. Effects of CXCL10 restoration on tumor angiogenesis and hypoxia. Restoration of CXCL10 expression suppresses tumor angiogenesis and reduces tumor hypoxia. (A) Hypoxic areas stained by anti-pimonidazole antibody. Quantified analysis was used image J software. (B) Effect of CXCL10 expression on Hypoxia-inducible factor-1α Staining section was photographed with a confocal microscope. Quantified analysis was used image J software. **p<0.001

IV. DISCUSSION

Angiogenesis is fundamental process for tumor growth, which has become a main target for anticancer therapies. A broad number of agents targeting both VEGF and its receptor have recently become standard treatments for renal cell carcinoma. Unfortunately, their efficacy is often restricted to a small number of tumor types and limited in time.[7] Thus, Mechanism of resistance to antiangiogenic agents (sunitinib, sorafenib, pazopanib and bevacizumab) have also been widely investigated in renal cell carcinoma. In previous studies, during treatment with antianiogenic agents, a great number of alternative proangiogenic factors can be upregulated and found in increased quantity in the plasma, also demonstrated an increase on circulating PIGF, VEGF, bFGF and PDGFB in various type of cancer during progression to bevacizumub.[7,21] IL-8 has been exhibited to be a pro-angiogenic factor that can mediate resistance to sunitinib in RCC in preclinical models.[22] Treatment with an anti-IL-8 antibody was able to restore sensitivity to sunitinib in resistance tumors.[7] Overexpression of genes, such as PRKX, TTBK2 and RSK, demonstrated to be connected to sunitinib resistance.[23] Therefore, PRKX knockdown and blockade of PRKX, TTBK2 and RSK could improve sensitivity to sunitinib. It was also found that sunitinib is able to block PGP and ABCG2 efflux pumps, which are responsible for drugs resistance.[24,25] As a results of this blockade, it is possible to restore sensitivity of RCC cells. However, at the same time, sunitinib trapping within these proteins reduced its therapeutic effects.[7]

At this point of time, restoring anti-angiogenic factors suppressed by tumor can be a therapeutic option to overcome the current resistance to anti-angiogenic therapies. We focused on CXCL10, one of CXC chemokine family, which recently is widely known anti- angiogenic factor. In this study, we found that CXCL10 inhibited proliferation and tube formation of HUVEC in a dose-dependent manner, while it did not affect in vitro tumor proliferation of renal cell carcinoma cells. These results suggest that CXCL10 is not direct tumor suppressor, but indirectly affects antitumor effect as anti-angiogenic

factor in renal cell carcinoma. We also observed expression of CXCL10 was downregulated in various renal carcinoma cell lines. Thus, we selected the kidney cancer-derived Caki-1 cell line, and generated stable overexpression of CXCL10 cell line for further study.

Overexpression of CXCL10 suppressed expression of pro-angiogenic factors including MMP9, EGF, VEGFB, VEGFC, HGF and HIF-1α in Caki-1 cells. The Vascular endothelial growth factor (VEGF) family and its receptors have been known a central, specific role in angiogenesis.[26] VEGF and its receptors mediate vascular permeability, endothelial proliferation, migration and survival.[27] Epithelial growth factor (EGF) has no direct effects on vascular endothelium, but is involved in tumor proliferation, metastasis, apoptosis, angiogenesis and wound healing.[27,28] Furthermore, proteolytic fragments of Hepatocyte growth factor (HGF) have potent anti-angiogenic effects not only inhibiting HGF-induced and VEGF-and bFGF-induced angiogenesis.[29] Particularly, Reduction of Hypoxia inducible factor-1 (HIF-1) that acts on the VEGF promoter was remarkable in CXCL10 overexpression cell line. These results indicate that CXCL10 is involved not only as an anti-angiogenic factor but also as a suppressor of critical angiogenic factors in renal cell carcinoma. In vivo Restoration of CXCL10 expression suppresses tumor growth in renal cell carcinoma by suppressing tumor angiogenesis in xenografts. In addition, CXCL10 successfully induced limited hypoxia in tumor cells despite of inhibiting tumor angiogenesis in renal cell carcinoma. However, mechanism for these results is unclear. It could be possible that CXCL10 is involved in vessel normalization by regulating other angiogenic factors including HIF1-α During treatment with an anti-angiogenic drug, the generation of an hypoxic condition may fuel tumor progression and treatment resistance. Therefore, combination treatment with anti-angiogenic drugs and inhibitors of hypoxia could be promising.[30] Our data suggest that CXCL10 is promising hypoxia inhibitor in renal cell carcinoma. Further studies are needed to define its role in hypoxia condition.

V. CONCLUSION

In summary, CXCL10 has a role as an anti-angiogneic factor and also as a suppressor of critical angiogenic factors in renal cell carcinoma. Restoration of CXCL10 expression suppresses tumor growth in renal cell carcinoma by impeding expression of other angiogenic factors including MMP9, EGF, VEGFB, VEGFC, HGF, HIF-1α in renal cell carcinoma as well as inducing anti-angiogenic effect on endothelial cells. Our data suggest that targeting CXCL10 might be a novel therapeutic option for advanced renal cell carcinoma.

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ABSTRACT(IN KOREAN)

신장암에서 CXCL10이 혈관생성에 미치는 영향

< 지도교수 홍 성 준 >

연세대학교 대학원 의과학과

진 현 아

혈관 생성과정은 여러 촉진인자와 억제인자가 함께 작용하는 복잡한 과정이다. 종양은 성장하는데 있어서 혈관생성촉진인자들과 혈관생성억제인자들의 불균형을 유도한다. CXC 케모카인 집단은 혈관생성촉진인자들과 혈관생성억제인자들로 구성되어있다. 최근 연구에서 CXC 케모카인 집단 중 하나인 C-X-C 케모카인 모티프 10 (CXCL10)이 bFGF, VEGF과 관련하여 혈관생성억제인자로써 작용한다는 것이 밝혀졌다. 그러나 암과 혈관생성에 있어서 CXCL10의 역할은 명확하게 밝혀진 바가 없다. 본 연구에서 우리는 CXCL10이 신장암에서 종양 혈관생성에서의 역할과 그것의 메커니즘에 대해서 규명하고자 하였다.

우리는 CXCL10이 혈관내피세포인 HUVEC 세포의 고리 형성과 증식을 억제하지만 그러나 신장암세포 주의 증식에는 영향이 없는 것을 확인하였으며 신장암 세포 주중 Caki-1, UMRC3, UMRC6 세포주에서 발현이 낮아져 있는 것을 관찰하였다. CXCL10의 과발현은 HIF-1α, EGF, VEGFB, VEGFC, HGF, MMP9 포함한 혈관생성촉진인자들의 발현을 억제한다. 또한 동물실험에서 CXCL10의 발현 회복은 종양 혈관생성을 제어함으로써 종양성장을 확연히 억제함을 보여주었다.

본 연구는 CXCL10이 신장암에서 직접적으로 혈관생성억제인자로 작용함과 동시에 주요 혈관생성촉진인자들의 작용을 억제한다는 것을 보여준다. 이것은 암 치료에 있어 CXCL10을 표적하는 것이 새로운

치료적 선택권이 될 수 있음을 시사한다.

핵심되는 말 : CXCL10, 종양혈관생성, 신장암