Gene Regulation:

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CXCR3-B Can Mediate Growth-inhibitory Signals in Human Renal Cancer Cells by Down-regulating the Expression of Heme Oxygenase-1*

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Dipak Datta[‡§1], **Pallavi Banerjee**[‡§], **Martin Gasser**[¶], **Ana Maria Waaga-Gasser**[¶], **and Soumitro Pal**[‡§2]

From the [‡]*Division of Nephrology, Children's Hospital, Boston, Massachusetts 02115, the* [§]*Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115, and the* [¶]*Department of Surgery I, Molecular Oncology and Immunology, University of Würzburg, 97080 Würzburg, Germany*

The chemokine receptor CXCR3 may play a critical role in the growth and metastasis of tumor cells, including renal tumors. It has been shown that CXCR3 has two splice variants with completely opposite functions; CXCR3-A promotes cell proliferation, whereas CXCR3-B inhibits cell growth. We recently demonstrated that the expression of growth-promoting CXCR3-A is up-regulated, and the growth-inhibitory CXCR3-B is markedly down-regulated in human renal cancer tissues; and the overexpression of CXCR3-B in renal cancer cells can significantly inhibit cell proliferation. However, the growth-inhibitory signal(s) through CXCR3-B are not well characterized. Here, we investigated the effector molecule(s) involved in CXCR3-B-mediated signaling events. We found that the overexpression of CXCR3-B in human renal cancer cells (Caki-1) promoted cellular apoptosis as observed by FACS analysis through Annexin-V staining. To examine whether the overexpression of CXCR3-B could alter the expression of any apoptosis-related genes in renal cancer cells, we performed a protein array. We found that CXCR3-B overexpression significantly down-regulated the expression of antiapoptotic heme oxygenase-1 (HO-1). By utilizing a HO-1 promoter-luciferase plasmid, we showed that CXCR3-B-mediated down-regulation of HO-1 was controlled at the transcriptional level as observed by luciferase assay. We also demonstrated that the inhibition of HO-1 expression using siRNA promoted apoptosis of renal cancer cells. Finally, we observed that human renal cancer tissues expressing low amounts of CXCR3-B significantly overexpress HO-1 at both mRNA and protein level. Together, we suggest that the overexpression of CXCR3-B may prevent the growth of renal tumors through the inhibition of antiapoptotic HO-1.

Chemokines are small cytokine-like secreted proteins that have chemoattractant properties and are well established to function in the recruitment of leukocytes into inflamed tissues (1, 2). The biologic effects of chemokines are mediated through specific G protein-coupled receptors (3). Besides their functions in the immune system, chemokines and chemokine receptors also play a critical role in tumor initiation, progression, and metastasis (4–6). Recent studies clearly suggest that in several malignancies, tumor cells express different chemokines and their receptors that are associated with more aggressive disease and poorer prognosis (7–12).

The chemokine receptor CXCR3 is classically expressed on T cells (3, 13); however, recent reports have shown that it may also be expressed in different types of tumor cells, including renal tumors (7, 9–11, 14, 15). CXCR3 can interact with its specific ligands, CXCL9, CXCL10, and CXCL11 (13). It has been reported that CXCR3 is alternatively spliced in different human tissues to produce two known variants, CXCR3-A and CXCR3-B (7, 13, 16–18). Some tissues, such as heart, kidney, liver, skeletal muscle, and human airway epithelial cells, express both splice variants, whereas other tissues, such as placenta and human mesangial cells, express only CXCR3-A, and human microvascular endothelial cells selectively express CXCR3-B. Each of the CXCR3 splice variants mediates completely different intracellular signals and has distinct functions (13, 16, 17). CXCR3-A promotes chemotaxis and cell proliferation, whereas CXCR3-B signals for growth inhibition (7, 16, 18, 19).

We have recently demonstrated that the expression of the growth-promoting CXCR3-A is markedly high in human renal cancer cells; in contrast, the expression of the growth-inhibitory CXCR3-B is significantly low in these cells (18). We suggested that in the absence/low presence of CXCR3-B, renal cancer cells undergo increased proliferation and migration through CXCR3-A-mediated signaling events. In support of this finding, we observed that the overexpression of CXCR3-B indeed inhibited renal cancer cell proliferation (18). Thus, it is likely that signaling through CXCR3-B in renal cancer cells may inhibit cell growth.

It is now established that the postreceptor signals via CXCR3-B result in an increase in intracellular cyclic AMP levels, whereas CXCR3-A mediates calcium influx within the cell (16, 19, 20). Although coupling of the G proteins to chemokine receptors is important for their biological activities, CXCR3-A and CXCR3-B may mediate their functions through different G protein coupling (7, 16, 19). Petrai *et al.* (21) showed that the growth-inhibitory signal through CXCR3-B is mediated by the p38 mitogen-activated protein kinase (MAPK) pathway. However, the effector molecules for CXCR3-B-mediated signaling events in terms of tumor growth/inhibition are not characterized.

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[1] Present address: Department of Pathology, Beth Israel Deaconess Medical Center, Boston, MA 02215.

[2] To whom correspondence should be addressed: Division of Nephrology, Children's Hospital, 300 Longwood Ave., Boston, MA 02115. Tel.: 617-919-2989; Fax: 617-730-0130; E-mail: soumitro.pal@childrens.harvard.edu.

Heme oxygenase-1 (HO-1)[2] is a rate-limiting enzyme in heme degradation, and it converts heme to biliverdin, carbon monoxide (CO), and iron (22–24). It plays an important protective role in the tissues by reducing oxidative injury, attenuating the inflammatory response, inhibiting cell apoptosis, and regulating cell proliferation (22, 25, 26). Although it is a cytoprotective enzyme, it has been reported that HO-1 activation may play a significant role in carcinogenesis and can influence the growth and metastasis of tumors (27–29). HO-1 is often highly up-regulated in tumor tissues, and the overexpressed HO-1 may promote tumor growth. It has been suggested that the inhibition of HO-1 expression can be a potential therapeutic approach to sensitize tumors to radiation and chemotherapy (30–32).

In this study, we show that HO-1 is significantly expressed in human renal cancer tissues and that the overexpression of CXCR3-B in human renal cancer cells can markedly down-regulate HO-1 expression at both transcriptional and protein levels. We also demonstrate that the overexpression of CXCR3-B and the inhibition of HO-1 can lead to augmented apoptosis of these cells. These observations suggest that CXCR3-B may mediate growth-inhibitory signal(s) in renal cancer cells through down-regulation of HO-1 expression.

EXPERIMENTAL PROCEDURES

Reagents—The small interfering RNA (siRNA) for HO-1 and its control were purchased from Qiagen. The transfection of siRNA was performed using Lipofectamine 2000 (Invitrogen). Cobalt protoporphyrin (CoPP) was obtained from Frontier Scientific.

Cell Line—The human renal cancer cell line Caki-1 was obtained from American Type Culture Collection. The cells were grown in McCoy's medium supplemented with 10% fetal bovine serum (Invitrogen).

Tissue Samples—Tissue samples of human renal cell cancer (RCC) were obtained from surgical specimens of patients who underwent surgery at the University Hospital (Würzburg, Germany). The protocol to obtain tissue samples was approved by the review board of the hospital. Tumor tissues were graded (stages I through IV) according to Robson staging system. Normal renal tissues were obtained from normal parts of the surgical specimens, and the normalcy of these tissues was confirmed by histology.

Plasmids—CXCR3-B overexpression plasmid (pcDNA3-CXCR3-B) was a gift from P. Romagnani (University of Florence, Florence, Italy) (21). The human HO-1 promoter-luciferase construct was a gift from J. Alam (Alton Ochsner Medical Foundation, New Orleans, LA). The plasmid phHO4luc was constructed by cloning the promoter fragment from the human HO-1 gene (bp −4067 to 70 relative to transcription start site) into the luciferase reporter gene vector pSKluc.

Flow Cytometry—To measure intracellular CXCR3 expression, the cells were fixed/permeabilized in Cytofix/Cytoperm solution (BD Biosciences) for 20 min at 4 °C and washed with Perm/Wash solution (BD Biosciences). The fixed cells were then incubated with FITC-conjugated antibody to total CXCR3 (R & D Systems) or matched IgG isotype for 30 min at 4 °C. The stained cells were analyzed in a FACSCalibur (Becton Dickinson).

RNA Isolation and Real-time PCR—Total RNA was prepared using the RNeasy isolation kit (Qiagen), and cDNA was synthesized using cloned avian myeloblastosis virus first-strand synthesis kit (Invitrogen). To analyze mRNA expression, we performed real-time PCR using the Assay-on-Demand Gene Expression product (TaqMan, Mammalian Gene Collection probes) according to the manufacturer's instructions (Applied Biosystems). As an internal control, 18 S mRNA was amplified and analyzed under identical conditions. Gene-specific primer-probe sets for human CXCR3-B/HO-1/18 S were obtained from Applied Biosystems. The *Ct* value (the cycle number at which emitted fluorescence exceeded an automatically determined threshold) for the gene of interest was corrected by the *Ct* value for 18 S and expressed as Δ*Ct*. The fold change of mRNA amount was calculated as follows: (fold changes) = 2^X (where $X = \Delta Ct$ for control group − ΔCt for experimental group).

Protein Array—Protein array was performed using apoptosis protein array kit from R & D Systems, according to the manufacturer's protocol.

Transfection and Luciferase Assay—Caki-1 (2.5×10^5 cells) were transfected with the CXCR3-B overexpression plasmid or the HO-1 promoter-luciferase plasmid using the Effectene transfection reagent (Qiagen), according to the manufacturer's protocol. The total amount of transfected plasmid DNA was normalized using a control empty expression vector. Transfection efficiency was determined by co-transfection of the β-galactosidase gene under control of cytomegalovirus immediate-early promoter and by measurement of β-galactosidase activity. For luciferase assay, cells were harvested 48 h after transfection, and luciferase activity was measured using a standard assay kit (Promega) in a luminometer.

Apoptosis Assay—The cellular apoptosis was measured by Annexin-V and propidium iodide staining using an FITC Annexin-V Apoptosis Detection kit (BD Pharmingen) according to the manufacturer's protocol. Following staining, the cells were analyzed by FACS analysis.

Western Blot Analysis—Protein samples were run on SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane (NEN Life Sciences Products). The membranes were incubated with anti-HO-1 (R & D Systems) or anti-β-actin (Santa Cruz Biotechnology) and subsequently incubated with peroxidase-linked secondary antibody. The reactive bands were detected by chemiluminescence (Pierce).

Immunohistochemistry—Immunohistochemistry was performed on frozen sections of human RCC tissues and normal renal tissues. Briefly, acetone-fixed sections were incubated first with anti-human HO-1 (Abcam) and second with a species-specific horseradish peroxidase-conjugated secondary antibody. Specimens were washed thoroughly in between incubations, developed in 3,3′-diaminobenzidine (Bio-Genex), and counterstained in Meyer's Hemalaun using standard techniques.

[2] The abbreviations used are: HO-1, heme oxygenase-1; CoPP, cobalt protoporphyrin; FACS, fluorescence-activated cell sorter; RCC, renal cell cancer; ROS, reactive oxygen species.

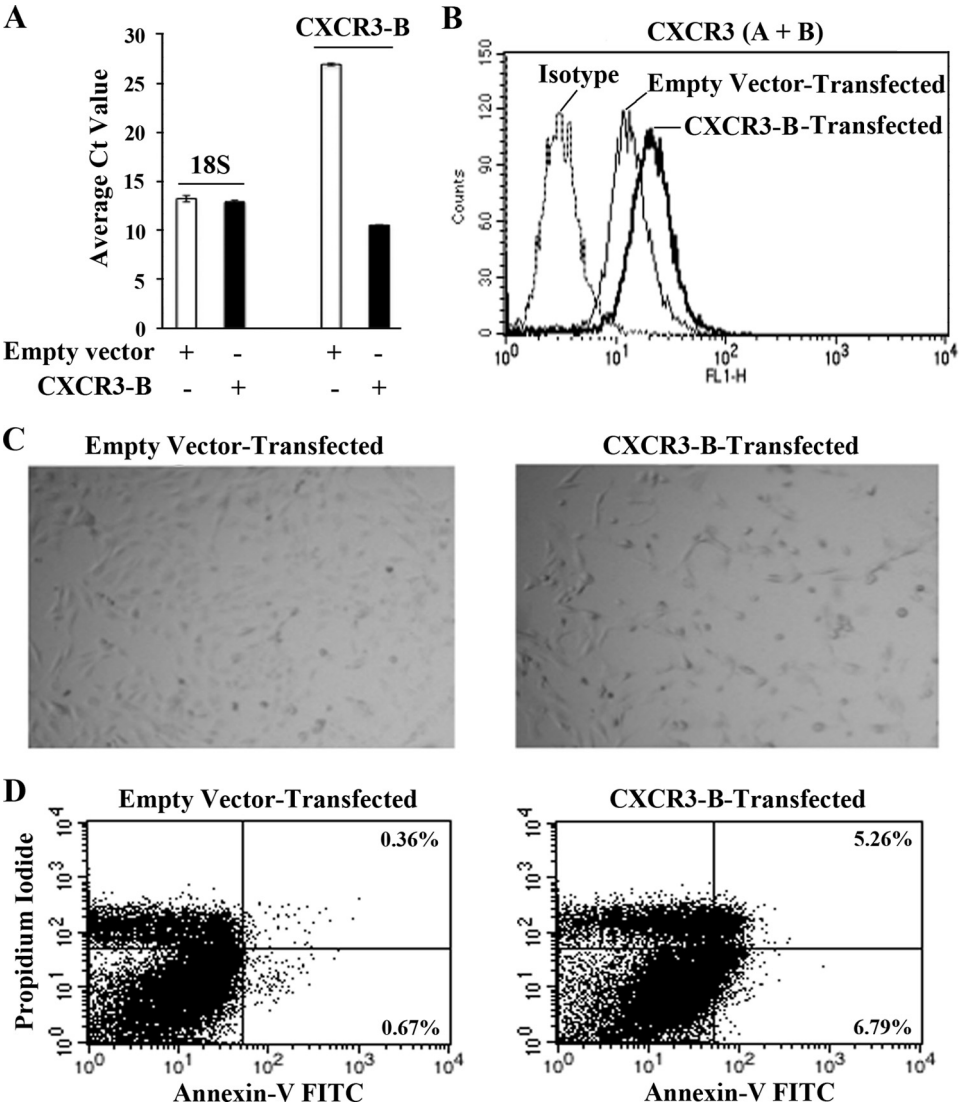


FIGURE 1. **Overexpression of CXCR3-B in human renal cancer cells promotes apoptosis.** Caki-1 cells were transfected with either the CXCR3-B overexpression plasmid (1.0 μg) or the empty expression vector. Following 48 h of transfection (*A*) the CXCR3-B mRNA expression was checked by real-time PCR (the lower *Ct* value reflects overexpression of the gene), and (*B*) the CXCR3 (A + B) protein expression was measured by FACS analysis. *C*, the confluence of the cells was evaluated microscopically. *D*, the apoptotic index of the cells was determined by Annexin-V and propidium iodide staining using an Apoptosis Detection kit as described under "Experimental Procedures." All results are representative of three independent experiments.

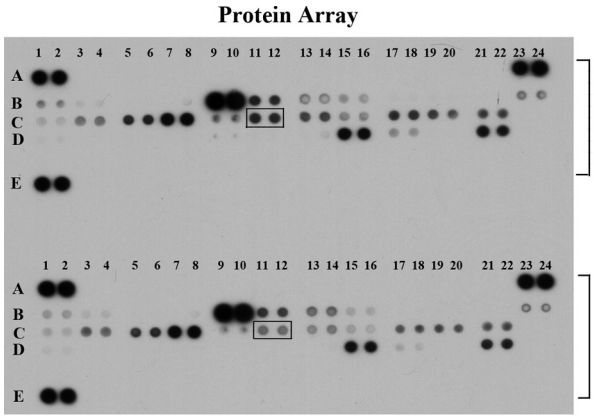
Statistical Analysis—Statistical evaluation for data analysis was determined by Student's *t* test. Differences of $p < 0.05$ were considered statistically significant.

RESULTS

Overexpression of CXCR3-B Promotes Apoptosis of Human Renal Cancer Cells—We first wished to examine whether the overexpression of CXCR3-B in human renal cancer cells could promote apoptosis. The Caki-1 cells were transfected with either the CXCR3-B overexpression plasmid or the empty vector. The overexpression of CXCR3-B in these cells was confirmed by real-time PCR, using gene-specific primer probe sets (Fig. 1*A*). We also confirmed the overexpression of CXCR3 protein by FACS analysis, using an antibody for total CXCR3 that recognizes both CXCR3-A and CXCR3-B (due to unavailability of specific antibody against CXCR3-B) (Fig. 1*B*). Following 48 h of transfection, we checked the confluence of these cells under the microscope. We observed that the empty vector-transfected cells were ~70% confluent; in contrast, CXCR3-B-transfected cells were only ~40% confluent (Fig 1*C*). This finding preliminarily suggests that CXCR3-B overexpression may mediate a growth-inhibitory effect in renal cancer cells.

To evaluate the apoptotic index of CXCR3-B-transfected cells, they were stained with Annexin-V and propidium iodide and subjected to FACS analysis. We observed that there was an increase in apoptotic cells following CXCR3-B overexpression, compared with empty vector-transfected controls (Fig. 1*D*); the percentage of early apoptotic cells increased from 0.67% (control cells) to 6.79%, and the late apoptotic cells increased from 0.36% (control cells) to 5.26%. Together, we suggest that the overexpressed CXCR3-B in human renal cancer cells may interact with CXCR3-binding ligands that are overexpressed in these cells as shown in our previous study (18); this interaction can lead to the generation of CXCR3-B-mediated negative signal(s) that may induce cellular apoptosis.

Overexpression of CXCR3-B Down-regulates HO-1 Expression in Renal Cancer Cells—We next questioned whether the overexpression of CXCR3-B could alter the expression of any apoptosis-related protein(s) in renal cancer cells. To this end, we performed a protein array utilizing an apoptosis array kit to examine the change in expression of 35 apoptosis-related genes. The Caki-1 cells were transfected with either the CXCR3-B overexpression plasmid or the empty vector. Following transfection (48 h), the cells were lysed, and the lysates were analyzed for the expression of apoptosis-related proteins as described above. As shown in the Fig. 2, the overexpression of CXCR3-B significantly down-regulated the expression of the antiapoptotic protein HO-1 compared with empty vector-transfected controls. There was also some down-regulation in the expression of HO-2 following CXCR3-B overexpression; however, there was no significant change in the expression of other proteins. It has been reported that both HO-1 and HO-2 are expressed in renal tissues; however, only HO-1, and not HO-2, is significantly elevated in renal cancer (29). This finding suggests that CXCR3-B may mediate a growth-inhibitory apo-



Coordinate	Target/Control	Coordinate	Target/Control
A1, A2	Positive Control	C13, C14	HO-2
A23, A24	Positive Control	C15, C16	HSP27
B1, B2	Bad	C17, C18	HSP60
B3, B4	Bax	C19, C20	HSP70
B5, B6	Bcl-2	C21, C22	HTRA2/Omi
B7, B8	Bcl-x	C23, C24	Livin
B9, B10	Pro-Caspase-3	D1, D2	PON2
B11, B12	Cleaved Caspase3	D3, D4	p21/CDNK1A
B13, B14	Catalase	D5, D6	p27Kip1
B15, B16	cIAP-1	D7, D8	phospho-p53 (S15)
B17, B18	cIAP-2	D9, D10	phospho-p53 (S46)
B19, B20	Claspin	D11, D12	phospho-p53 (S392)
B21, B22	Clusterin	D13, D14	phospho-Rad17
B23, B24	Cytochrome c	D15, D16	SMAC/Diablo
C1, C2	TRAIL R1/DR4	D17, D18	Survivin
C3, C4	TRAIL R2/DR5	D19, D20	TNFR1/TNFRSF1A
C5, C6	FADD	D21, D22	XIAP
C7, C8	Fas/TNFSF6	D23, D24	PBS (-ve Control)
C9, C10	HIF-1α	E1, E2	Positive Control
C11, C12	HO-1		

FIGURE 2. **Overexpression of CXCR3-B in renal cancer cells inhibits the expression of antiapoptotic HO-1.** Caki-1 cells were transfected with either the CXCR3-B overexpression plasmid (1. 0 μg) or the empty expression vector for 48 h. The cells were lysed, and a protein array was performed utilizing an apoptosis protein array kit. The *table chart* beside the *blot* indicates the individual proteins tested in this array. Results are representative of three independent experiments.

ptotic signal in human renal cancer cells through the down-regulation of antiapoptotic HO-1, which is the inducible isoform of heme oxygenase.

CXCR3-B Overexpression Down-regulates HO-1 Promoter Activity—As our earlier experiment suggested that the overexpression of CXCR3-B can inhibit the expression of HO-1 in renal cancer cells, we next sought to determine whether CXCR3-B could regulate HO-1 promoter activity. The Caki-1 cells were co-transfected with the HO-1 promoter-luciferase construct and either the CXCR3-B overexpression plasmid or the empty vector. The effect of CXCR3-B overexpression on HO-1 promoter activation was assessed by the measurement of luciferase activity in cell lysates. As shown in Fig. 3*A*, CXCR3-B overexpression significantly inhibited HO-1 promoter activity.

We also confirmed through Western blot analysis that CXCR3-B-mediated down-regulation of HO-1 promoter activity was associated with a decrease in HO-1 at the protein level. As shown in Fig. 3*B*, transfection with the CXCR3-B overexpression plasmid markedly inhibited HO-1 protein expression in Caki-1 cells, compared with empty vector-transfected controls. Together, these experiments suggest that CXCR3-B can inhibit the expression of antiapoptotic HO-1 by down-regulating its promoter activity.

Down-regulation of HO-1 Is Associated with Increased Apoptosis of Renal Cancer Cells—We next set out to determine whether the down-regulation of HO-1 expression could promote apoptosis in renal cancer cells. To knock down HO-1, the Caki-1 cells were transfected with either HO-1 siRNA or control siRNA. Following transfection with HO-1 siRNA, we performed the apoptosis assay through Annexin-V and propidium iodide staining, and the cells were analyzed by FACS analysis as described earlier. As shown in Fig. 4*A*, we observed that the knockdown of HO-1 in renal cancer cells increased cellular apoptosis compared with control cells; the percentage of early apoptotic cells increased from 0.93% (control cells) to 5.28%. The knockdown of HO-1 was confirmed by Western blot analysis (Fig. 4*B*).

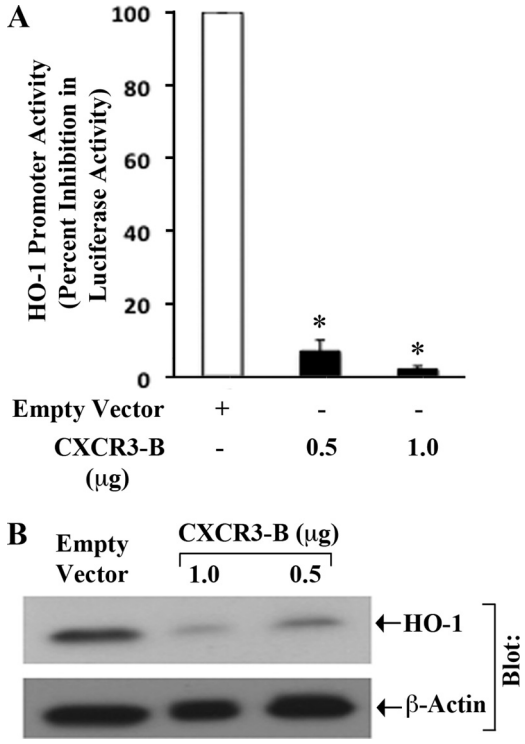


FIGURE 3. **Overexpression of CXCR3-B inhibits HO-1 promoter activity.** *A*, Caki-1 cells were co-transfected with the HO-1 promoter-luciferase construct (1.0 μg) and either different amounts (0.5 and 1.0 μg) of CXCR3-B overexpression plasmid (*filled columns*) or the empty expression vector (*open column*). The cells were harvested after 48 h, and the percent decrease in luciferase activity was calculated from luciferase counts of each group of cells compared with that of cells transfected with empty expression vector alone. Results are representative of three independent experiments. *Columns*, average of triplicate readings of samples; *error bars*, ± S.D. *, $p < 0.01$, compared with empty vector-transfected cells. *B*, Caki-1 cells were transfected with either different amounts (0.5 and 1.0 μg) of CXCR3-B overexpression plasmid or the empty expression vector. Following 48 h of transfection, whole cell lysates were prepared, and Western blot analysis was performed using anti-HO-1 to quantitate HO-1 protein expression. The expression of β-actin in these cells was analyzed by Western blotting using anti-β-actin. Results are representative of three independent experiments.

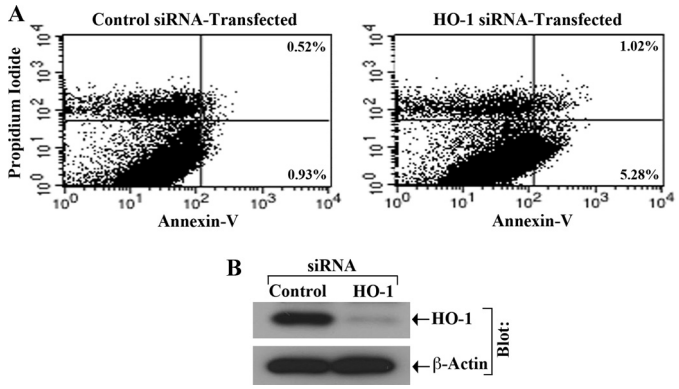


FIGURE 4. **Down-regulation of HO-1 promotes apoptosis of renal cancer cells.** Caki-1 cells were transfected with either the control or the HO-1 siRNA (50 nM) and cultured for 48 h. *A*, the apoptotic index of the cells was determined by Annexin-V and propidium iodide staining using an Apoptosis Detection kit as described under "Experimental Procedures." *B*, the knockdown of HO-1 was confirmed by Western blot analysis. Results are representative of three independent experiments.

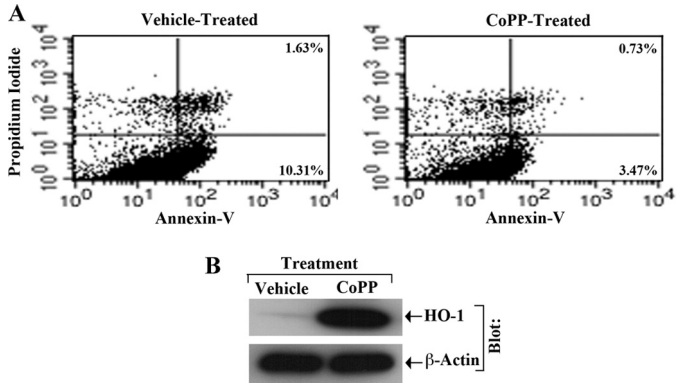


FIGURE 5. **Overexpression of HO-1 inhibits apoptosis of renal cancer cells.** Caki-1 cells were treated with either CoPP (50 μM) or the vehicle alone for 48 h. *A*, apoptotic index of the cells was determined by Annexin-V and propidium iodide staining using an Apoptosis Detection kit as described under "Experimental Procedures." *B*, overexpression of HO-1 following CoPP treatment was confirmed by Western blot analysis. Results are representative of three independent experiments.

We also examined the apoptotic index of these renal cancer cells following overexpression of HO-1 through CoPP treatment. As shown in Fig. 5*A*, we observed that the CoPP treatment significantly inhibited cellular apoptosis compared with vehicle-treated control cells; the percentage of early apoptotic cells decreased from 10.31% (vehicle-treated cells) to 3.47%. The overexpression of HO-1 in CoPP-treated cells was confirmed by Western blot analysis (Fig. 5*B*). Taken together, the data suggest that HO-1 may mediate a survival signal in human renal cancer cells and the down-regulation of its expression may lead to cellular apoptosis.

HO-1 Is Significantly Up-regulated in Human Renal Cancer Tissues Expressing Low Levels of CXCR3-B—In our previous study (18), we have demonstrated that the expression of CXCR3-A is highly up-regulated, whereas CXCR3-B is markedly down-regulated in human RCC tissues compared with normal renal tissues. To study the pathophysiological significance of the CXCR3-B–HO-1 axis, here we examined the expression of HO-1 in these RCC tissues. Through real-time PCR, we observed that HO-1 was overexpressed in all the RCC

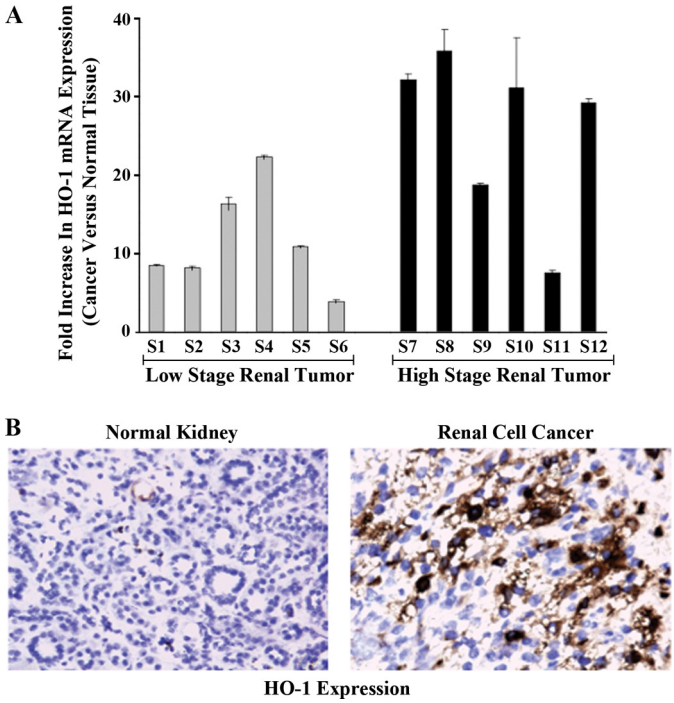


FIGURE 6. **HO-1 is overexpressed in human renal cancer tissues.** *A*, total RNA was isolated from renal cancer and normal renal tissues and reverse transcribed. The fold increase of HO-1 mRNA expression in renal cancer tissues *versus* normal renal tissues was measured by real-time PCR. *S1–S6* represent low stage (Robson stages I and II), whereas *S7–S12* represent high stage (Robson stages III and IV) renal tumor tissues. *Columns* are average of triplicate readings of the samples; *error bars* are ± S.D. *B*, representative photomicrographs show the expression of HO-1 in human renal cell cancer and normal kidney tissues detected by immunohistochemistry. Magnification, ×400. *Brown color*, expression of HO-1. Results are representative of three different tissues.

tissues (both low stage and high stage tumors), compared with normal renal tissues (Fig 6*A*).

We also checked the expression of HO-1 in RCC tissue through immunohistochemistry. As shown in Fig. 6*B*, HO-1 was significantly expressed in renal cancer tissue compared with normal tissue. Together, we suggest that the low expression of CXCR3-B might be associated with a significantly high HO-1 expression in human renal cancer tissues.

DISCUSSION

The two splice variants of the chemokine receptor CXCR3 may have completely opposite functions in different cell types; CXCR3-A may promote cell proliferation, whereas CXCR3-B may mediate growth inhibition (7, 16, 19). In the present study, we show that the overexpression of CXCR3-B in human renal cancer cells can mediate a growth-inhibitory effect through the down-regulation of antiapoptotic HO-1 expression.

Reactive oxygen species (ROS), such as superoxide anion radicals and hydrogen peroxide, are potentially harmful by-products of normal cellular metabolism that directly affects cellular function (30, 33). An increased level of ROS may promote significant damage to cell structure and functions (34). HO-1, a member of the heat shock protein family, plays a key role as a sensor and regulator of cellular oxidative stresses (35, 36). HO-1 is induced in tissues during these stress conditions, and it promotes cytoprotection. On the other hand, it has been

demonstrated that HO-1 is overexpressed in different types of human tumors (27–29), and this enzyme can facilitate tumor progression through different mechanism(s). It has been suggested that HO-1 may be used as one of the potential targets in antitumor therapy to increase the level of ROS within the tumor and promote effective killing of the tumor cells (30, 32). Many antitumor agents, such as vinblastine, cisplatin, mitomycin C, doxorubicin, camptothecin, inostamycin, and neocarzinostatin, exhibit antitumor activity via ROS-dependent activation of apoptotic cell death (37).

We have previously demonstrated that the expression of growth-promoting CXCR3-A is significantly high, whereas the expression of growth-inhibitory CXCR3-B is markedly low in human renal cancer tissues; and the overexpression of CXCR3-B can inhibit renal cancer cell proliferation (18). In this study, we demonstrate a novel mechanism in which the overexpression of CXCR3-B may mediate a growth-inhibitory effect through the inhibition of anti-apoptotic HO-1, which is significantly up-regulated in human renal cancer. We have shown that the renal cancer cells express CXCR3-binding ligands (18), and we suggest that these ligands may interact with overexpressed CXCR3-B in an autocrine manner to mediate apoptotic signals, including the inhibition of HO-1 expression. CXCR3-B-mediated down-regulation of HO-1 has been found to be regulated at the transcriptional level. It is expected that the inhibition of HO-1 by CXCR3-B mediate an apoptotic signal in human renal cancer cells through the elevation of ROS level.

It has been reported that the growth-inhibitory effect of CXCR3-B is mediated through the p38 MAPK signaling pathway (21). Keum *et al.* (38) have demonstrated that the activation of the p38 MAPK may down-regulate the expression of HO-1 in human cancer cells; and p38 MAPK-mediated inhibition of HO-1 expression is regulated at the transcriptional level. Thus, it is possible that p38 MAPK may act as one of the critical intermediary molecules in CXCR3-B-mediated signaling events for HO-1 inhibition. However, we cannot rule out the role(s) of other intermediary molecules, as the growth-inhibitory signaling event(s) through CXCR3-B is a complex process and needs to be elucidated. It is also possible that CXCR3-B-mediated down-regulation of HO-1 is an indirect response through the inhibition of CXCR3-A-mediated signaling pathway(s).

In summary, we suggest that HO-1 may act as a therapeutic target in human renal cancer. The inhibition of HO-1 may allow the generation of ROS, and the elevated ROS level may facilitate tumor killing. Our observations in this study clearly show that the overexpression of CXCR3-B in renal cancer cells can down-regulate HO-1 expression, and mediate tumor cell apoptosis. Thus, the therapeutic strategies that may induce CXCR3-B-mediated signaling events(s) may prevent/restrict the growth of human renal cell cancer through the inhibition of HO-1 expression.

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