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# Prognostic value of CXCR4 expression in patients with clear cell renal cell carcinoma

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**Summary.** Introduction: The expression of CXCR4 is implicated in the metastatic dissemination of different cancers. The information on its prognostic value has been very limited in clear cell renal cell carcinoma (ccRCC). Our objective was to explore the prognostic value of CXCR4 in ccRCC. Materials and methods: 104 patients with a ccRCC were studied. There were 69 men and 35 women with an average age of 64.5 years old (range: 34-86 years). The CXCR4 expression was evaluated by immunohistochemistry. The follow-up varied from 12 to 184 months with a mean of 79.5 months. Kaplan-Meier with a log rank test was performed to compare overall survival and cancer-specific survival after surgery. Univariate and multivariate analyses were performed according to the Cox regression model. Results: CXCR4 expression was found in 68/104 (65.4%) of tumor samples. CXCR4 expression was located in the nucleus in 55/68 (80.8%) cases while cytoplasm or membrane location was found in 13/68 (19.2%) cases. High expression was found in 25/68 (36.8%) cases. During follow-up, 39 patients died, of which 26 died of cancer. Kaplan-Meier analysis revealed that a high expression of CXCR4 was associated with a reduced overall survival (p=0.017) and cancer-specific survival (p=0.022). Univariate analysis indicated that a high expression of CXCR4 was a significant factor for a poorer overall survival (p=0.020) and cancer-specific survival (p=0.027). By multivariate analysis, a high expression of CXCR4 appeared to be an independent factor of overall survival (p=0.024) and cancer-specific survival (p=0.028). Conclusion: This study suggested that a high CXCR4 expression was correlated with a worse outcome for ccRCC patients.

**Key words:** CXCR4, Prognosis, Renal cell carcinoma, Immunohistochemistry

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## Introduction

Renal cell carcinoma is increasing steadily. Despite the fact that small tumors are found frequently, one third of patients with renal cancer present a metastatic disease at the time of diagnosis and 30-40% of patients with localized renal cancer will develop a metastasis after surgery (Lam et al., 2005). Currently, the standard treatment consists of radical or partial nephrectomy. Metastatic renal cell carcinoma is generally resistant to chemotherapy and hormonal therapy. The medical treatment for metastatic renal cancer is immunotherapy with a 10% response rate. 40% of patients with renal cell carcinoma will die from the metastasis (Kim et al., 2011). These data indicate that prognostic prediction is a major issue for patients' follow-up. At present, the prediction of prognosis relies only on the clinical stage and grade. Efficient prognostic markers are urgently needed to guide surveillance.

Like other cancers, the processes of progression and metastasis in renal cell carcinoma include complicated molecular interactions. Many pathways are involved in these processes. The human chemokine system is currently known to include more than 40 chemokines.

Chemokines exert many functions, such as induction of cell differentiation, proliferation and directional migration of several cell types (Richard and Blay, 2008). Chemokine receptors are a family of seven transmembrane G protein-coupled cell surface receptors. There are 19 well-recognized chemokine receptors. CXCR4 belongs to the family of chemokine receptors and is the best studied chmokine receptor in cancer due to its ability to mediate the metastasis of various cancers (Richard and Blay, 2008). It has been demonstrated as a potential biomarker in several cancers (Richard and Blay, 2008; Furusato et al., 2010).

Clear cell renal cell carcinoma (ccRCC) is the most frequent subtype of renal cell carcinoma. It is characterized by the inactivation of the von Hippel Landau (VHL) gene. Since 2003, the role of CXCR4 in ccRCC has been studied (Staller et al., 2003). The loss of VHL protein incites the increase of CXCR4 in ccRCC (Staller et al., 2003). Several models have shown evidence that CXCR4 participates in the metastasis of ccRCC (Zagzag et al., 2005; Pan et al., 2006; Wang et al., 2009). However, there has been scarce information concerning its prognostic value in ccRCC. Therefore, we conducted a clinical study to explore its prognostic value in ccRCC.

## Materials and methods

### *Patient characteristics and tumor samples*

104 patients with ccRCC were included. Patients were treated between 1999 and 2005. There were 69 men and 35 women with an average age of 64.5 years old (range: 34-86 years). The treatment included either a radical or partial nephrectomy. The tumors were staged according to the TNM classification and graded according to the Fuhrman criteria (Fuhrman et al., 1982; Edge et al., 2010 ). All patients were followed up regularly after surgery. Briefly, patients had one visit every three months during the first two years after surgery; two visits every six months from the third year to the fifth year after surgery, and then one visit per year. The surveillance included image examination, biological examination and physical examination.

The patients' stage was: 48 pT1, 8 pT2 and 48 pT3. The grade was: 17 G1, 46 G2, 34 G3 and 7 G4. There were 16 M+ and 4 N+. The ECOG status was: 77 patients for 0; 24 patients for 1; 2 patients for 2 and 1 patient for 3. Necrosis was found in 45 tumor samples while 59 tumors were absent of necrosis.

After a mean follow-up of 79.5 months, 39 deaths occurred. 26 deaths were due to the cancer.

The histological slides were re-examined by an experienced pathologist for diagnosis. The corresponding blocks were used for the immuhistochemical study.

A consent statement was obtained from each patient for the future study of tumor tissues when the patient was hospitalized. This research was approved by local ethics committee.

### *Immunohistochemical staining for CXCR4*

Immunohistochemical staining was performed according to our previous study with some modifications (Li et al., 2007). Paraffin-embedded, 5-$\mu$m-thick tissue sections from all primary tumors were stained for the CXCR4 protein using a primary rabbit polyclonal antibody (Abcam, ab74012; Cambridge, UK). Slides were deparaffinized through a series of xylene baths. Rehydration was performed through graded alcohols. Endogenous peroxidase activity was blocked in ChemMate Peroxidase Blocking solution (DakoCytomation) three times for 5 minutes for each time. The sections were then passed through the automate (LEICA BOND III, Microsystems) for a standard immunohistochemical protocol. Diaminobenzidine was used as a chromogen, and commercial hematoxylin was used for counterstaining. For each immunohistochemical staining, a positive and a negative control were used. CXCR4 expression was evaluated at the cytoplasmic, membrane and nuclear level. We used the range of positive cells to define an expression level: high ≥85%; moderate 25-85%; low <25% and absence =0%. The cut-off level for high expression was based on the published immunohistochemical study of renal cancer and our previous work (Li et al., 2007; Sandlund et al., 2007).

Immunohistochemical analysis was performed in a blinded manner with respect to the clinical data concerning the subjects.

### *Statistical analysis*

Survival was calculated by the Kaplan-Meier method, and the resulting curves were compared using the log-rank test. Non parametrical Mann-Withney test and $\chi^2$ test were used to analyze the association between two categorical variables. Univariate and multivariate analyses were based on the Cox regression model. The variables with $p<0.2$ in the univariate analysis were selected to be analyzed in the multivariate analysis. $p<0.05$ was considered to be statistically significant.

## Results

### *CXCR4 expression in ccRCC*

CXCR4 was found in 68/104 tumor samples. CXCR4 expression was located in nucleus in 55/68 (80.8%) cases while cytoplasm or membrane location was in 13/68 (19.2%) cases (Fig. 1). Co-expression of cytoplasm and membrane could be seen. Nucleus location was the major expression. Among the positive staining of CXCR4, high, moderate and low expression was found respectively in 25/68 (36.8%), 32/68 (47.1%)

and 11/68 (16.1%) cases.

### *CXCR4 expression: correlation with pathological parameters and outcome*

Kaplan-Meier analysis of overall survival and cancer-specific survival is shown in Figs. 2, 3. Fig. 2 revealed that a high expression of CXCR4 was associated with a reduced overall survival (p=0.017). Fig. 3 showed that a high expression of CXCR4 was associated with a poor cancer-specific survival (p=0.022). Univariate analysis was summed up in table 1. Age was a prognostic factor for overall survival (p=0.001), but not for cancer-specific survival (p=0.091). Sex was neither a prognostic factor for overall survival (p=0.779), nor for cancer-specific survival (p=0.504). TNM classification was a strong prognostic factor for cancer-specific survival (p<0.001 for T; N and M respectively). The Fuhrman grade was an important factor for cancer-specific survival (p<0.001). As expected, the ECOG or necrosis classification demonstrated its strong power in cancer-specific survival (p=0.001 or p=0.002 respectively). By univariate analysis, a high expression of CXCR4 was associated significantly with overall survival (p=0.020) and cancer-specific survival (p=0.027). Table 2 summarizes the results of multivariate analysis. By multivariate analysis, a high expression of CXCR4 appeared to be an independent factor of overall survival (p=0.024) and cancer-specific survival (p=0.028). However, the

**Table 1.** Univariate analysis.

| | | Overall survival | | | Cancer-specific survival | | |
|---|---|---|---|---|---|---|---|
| | Variable | HR | 95% CI | P | HR | 95% CI | P |
| Age | ≤67 | 1 | | | 1 | | |
| | >67 | 2.984 | 1.531-5.816 | 0.001 | 1.958 | 0.899-4.267 | 0.091 |
| Sex | Female | 1 | | | 1 | | |
| | Male | 1.102 | 0.558-2.178 | 0.779 | 1.344 | 0.565-3.197 | 0.504 |
| Stage | pT1-pT2 | 1 | | | 1 | | |
| | pT3 | 3.742 | 1.860-7.527 | <0.001 | 6.011 | 2.263-15.969 | <0.001 |
| pN | pN0 | 1 | | | 1 | | |
| | PN+ | 11.613 | 3.822-35 .287 | <0.001 | 15.170 | 4.787-48.076 | <0.001 |
| pM | M0 | 1 | | | 1 | | |
| | M+ | 3.351 | 1.622-6.922 | 0.001 | 5.647 | 2.551-12.500 | <0.001 |
| Fuhrman | 1-2 | 1 | | | 1 | | |
| | 3-4 | 2.530 | 1.341-4.773 | 0.004 | 5.184 | 2.75-12.353 | <0.001 |
| ECOG Status | 0 | 1 | | | 1 | | |
| | 1-3 | 2.833 | 1.205-6.689 | 0.017 | 5.209 | 1.892-14.342 | 0.001 |
| Necrosis | Absent | 1 | | | 1 | | |
| | Present | 4.076 | 1.580-10.514 | 0.004 | 7.034 | 2.003-24.305 | 0.002 |
| CXCR4 | ≥85% | 1 | | | 1 | | |
| | <85% | 2.182 | 1.132-4.204 | 0.020 | 2.445 | 1.109-5.390 | 0.027 |

HR: Hazard ratio; CI: Confidential interval; ECOG: Eastern cooperative oncology group.

**Table 2.** Multivariate analysis.

| | Overall survival | | | Cancer-specific survival | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | P | HR | 95% CI | P |
| Age | 2.741 | 1.389-5.410 | 0.004 | | | 0.286 |
| pT | 2.876 | 1.391-5.944 | 0.004 | 3.993 | 1.430-11.147 | 0.008 |
| PN | 11.216 | 3.411-36.873 | 0.001 | 8.632 | 2.422-30.793 | 0.001 |
| pM | | | 0.492 | 2.424 | 1.029-5.713 | 0.043 |
| Fuhrman | | | 0.775 | | | 0.264 |
| ECOG Status | | | 0.058 | 4.038 | 1.455-11.207 | 0.008 |
| Necrosis | 3.614 | 1.387-9.414 | 0.009 | 5.726 | 1.614-20.311 | 0.007 |
| CXCR4 | 2.206 | 1.110-4.380 | 0.024 | 2.601 | 1.109-6.101 | 0.028 |

HR: Hazard ratio; CI: Confidential interval; ECOG: Eastern cooperative oncology group.

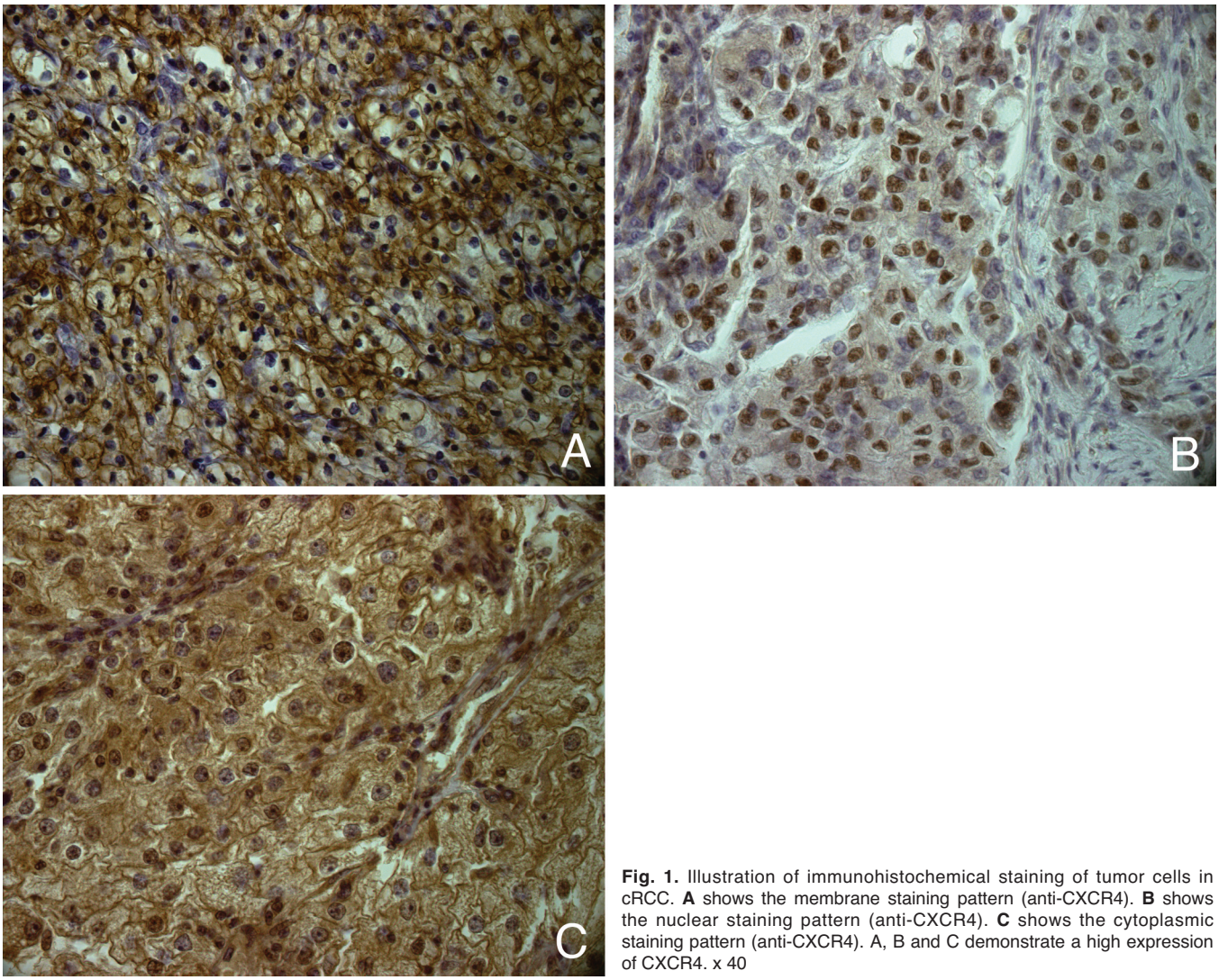


**Fig. 1.** Illustration of immunohistochemical staining of tumor cells in cRCC. **A** shows the membrane staining pattern (anti-CXCR4). **B** shows the nuclear staining pattern (anti-CXCR4). **C** shows the cytoplasmic staining pattern (anti-CXCR4). A, B and C demonstrate a high expression of CXCR4. x 40

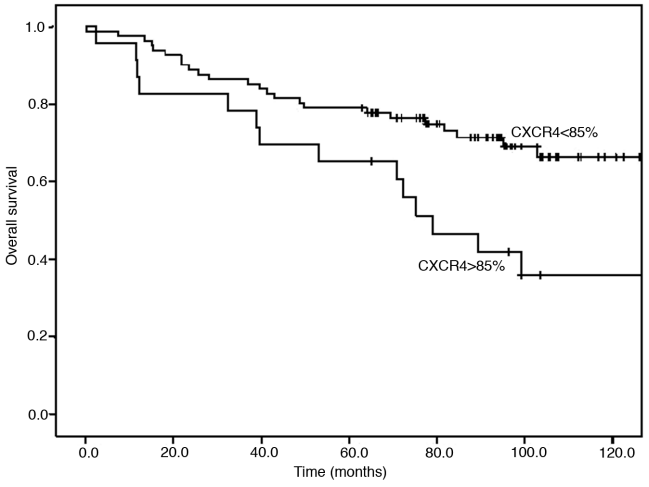


**Fig. 2.** Overall survival according to CXCR4 expression. A high expression of CXCR4 was associated with a reduced overall survival (p=0.017, log-rank test).

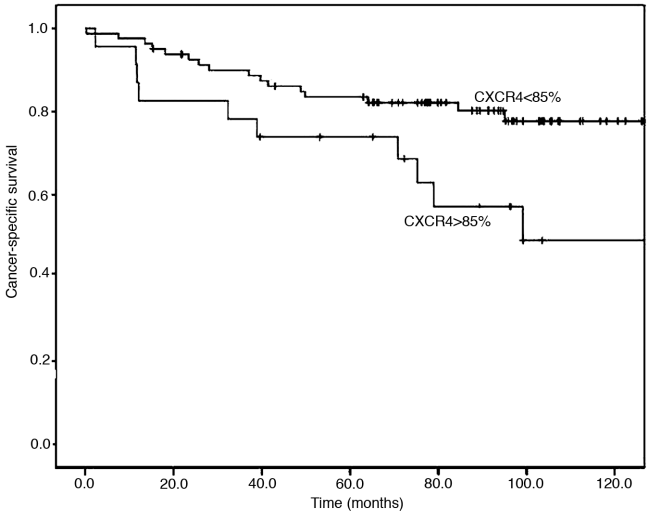


**Fig. 3.** Cancer-specific survival according to CXCR4 expression. A high expression of CXCR4 was associated with a poor cancer-specific survival (p=0.022, log-rank test).

Fuhrman grade lost its prognostic value for cancer-specific survival in the multivariate analysis. We associated grade 1 with grade 2 and grade 3 with grade 4. This association could have some effects on statistical analysis.

## Discussion

In the present study, we demonstrated that CXCR4 was located mainly in the nucleus of tumor cells, and that a high CXCR4 expression was correlated with worse survival.

CXCR4 has been found to be upregulated in many cancers (Richard and Blay, 2008; Furusato et al., 2010). Immunochemical study of CXCR4 in ccRCC has been rare. A previous study showed that CXCR4 expression was mainly in cytolplasma or membrance of tumor cells of ccRCC (Wehler et al., 2008). A striking finding was the presence of CXCR4 in the nucleus in 52.9% of the tumor samples of patients included in our series. CXCR4 expression has been described to be present either on the cell membrane or in the cytoplasm or in the nucleu (Furusato et al., 2010). The mechanism of CXCR4 expression in ccRCC is related to the VHL gene. The protein pVHL regulates negatively CXCR4 expression owing to its capacity to target hypoxia-inducible factor (HIF) for degradation under normoxic conditions (Staller et al., 2003). This process is suppressed under hypoxic conditions, resulting in HIF-dependent CXCR4 activation. Several experiments in cell line model have demonstrated the importance of signaling of CXCR4 in renal cancer cells with a prominent stimulation of genes involved in cell-cycle regulation and apoptosis (Schrader et al., 2002; Pan et al., 2006). CXCR4 may be crucial in controlling cell adhesion via its interactions with integrin receptors (Jones et al., 2007). It was suggested that CXCR4 signaling was a major mechanism of metastasis in ccRCC (Gahan et al., 2012). Taken together, we think that CXCR4 signaling is a major event in pVHL-HIF pathway that may participate in malignant transformation and progression of ccRCC.

The prediction of prognosis is based only on the pathological stage and grade. The biomarkers are urgently needed to guide for patient surveillance after surgery. Unfortunately, there have been no biomarkers that can be used in clinic of ccRCC. Since CXCR4 plays a major role in the process of metastasis of cancers, it is reasonable to explore its prognostic value in ccRCC. We found that a high expression of CXCR4 was a predictive factor of cancer-specific survival as well as overall survival. Furthermore, the predictive value was independent from the pathological parameters. The prognostic value of CXCR4 expression in ccRCC is scarce in literature. Only two studies are available in renal cancer. Our results support the findings by Stallet et al. (2003). In that study, they used a tissue microarray technique and found a striking positive correlation between strong CXCR4 expression and poor tumor-specific survival, while CA9 did not provide prognostic value on tumor-specific survival (Staller et al., 2003). D'Alterio et al demonstrated that CXCR4 expression was associated with disease free survival in renal cancer (D'Alterio et al., 2010). Besides, many articles have been recently published on the prognostic value of CXCR4 in other cancers (Spano et al., 2004; Furusato et al., 2010). It seems that a high expression of CXCR4 is associated with a poor outcome of patients of various cancers. We believe that CXCR4 is a new promising prognostic marker in ccRCC.

Another clinical implication of CXCR4 signaling in ccRCC may be its therapeutical potential (Haviv et al., 2004). Since 2006, molecular targeted therapy has changed the treatment strategy of metastatic renal cancer. The targeted therapy showed better results over the traditional immunotherapy in terms of reponse rate (Motzer et al., 2009). At present, there are six targeted agents approved for treatment in metastatic renal carcinoma: sorafenib, sunitinib, pazopanib, bevacizumab (in combination with interferon alpha), temsirolimus, and everolimus (Coppin et al., 2011). CXCR4 pathway provides a new cancer targeted therapy (Furusato et al., 2010). Serveral clinical trials are underway. Therefore, CXCR4 may be a new target for the treatment of metastatic ccRCC patients.

In conclusion, we found that CXCR4 was located principally in the nucleus of tumor cells in ccRCC. A high expression of CXCR4 was a predictive factor of worse outcome. The CXCR4 expression level may be used to predict the prognosis of ccRCC patients. More studies are needed to confirm our results.

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