Research Article

Strong Expression of Chemokine Receptor CXCR4 by Renal Cell Carcinoma Correlates with Advanced Disease

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Diverse chemokines and their receptors have been associated with tumor growth, tumor dissemination, and local immune escape. In different tumor entities, the level of chemokine receptor CXCR4 expression has been linked with tumor progression and decreased survival. The aim of this study was to evaluate the influence of CXCR4 expression on the progression of human renal cell carcinoma. CXCR4 expression of renal cell carcinoma was assessed by immunohistochemistry in 113 patients. Intensity of CXCR4 expression was correlated with both tumor and patient characteristics. Human renal cell carcinoma revealed variable intensities of CXCR4 expression. Strong CXCR4 expression of renal cell carcinoma was significantly associated with advanced T-status (P = .039), tumor dedifferentiation (P = .0005), and low hemoglobin (P = .039). In summary, strong CXCR4 expression was significantly associated with advanced dedifferentiated renal cell carcinoma.

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1. Introduction

Renal cell carcinoma (RCC) is the sixth leading cause of cancer-related deaths in the Western world and comprises 2–3% of all newly diagnosed malignancies in adults. Among the different kidney neoplasms, it represents with 85% the largest fraction [1]. The age-adjusted incidence of RCC in Western nations is 5–12/100 000 in women or men, respectively, with a peak incidence in the 6th decade [2]. In practice, the only curable treatment is nephrectomy performed in early stages of the disease. However, about 30–50% of patients have already metastases at presentation, and approximately one third of the nephrectomized patients relapse and progress with metastatic disease. The preferential sites of metastasis are the regional lymph nodes, the lung, the liver, and the bones. Survival strongly depends on the tumor stage at presentation. The 5-year survival rate is approximately 50%, whereas the median survival in case of metastasis is less than one year [3–5]. The current standard treatment for metastasized RCC consists of the application of IFN-α and IL-2 [6]. Recently, phase II clinical trials using receptor-tyrosine kinase (RTK) inhibitors have shown more promising results and lead to approval by the Food and Drug Administration (FDA) and European Medicines Agency (EMEA) [2].

In vivo and in vitro results from different tumor entities suggest that organ-specific metastasis is partially governed by interactions of chemokine receptors on cancer cells and their corresponding chemokines expressed in target organs and the tumor bed. This process is considered to direct lymphatic
and hematogenous spread and furthermore influences the
sites of metastatic growth [7]. Chemokines and their respec-
tive G-protein-coupled receptors were initially described to
mediate different pro- and anti-inflammatory responses [8].
In particular, the high expression of stromal cell derived
factor 1α (SDF-1α), also known as CXCL12, by endothelial
cells, biliary epithelial cells, bone marrow stromal cells, and
lymph nodes results in a chemotactic gradient attracting
CXCR4 expressing lymphocytes into those organs [9–15].
Most recently, CXCR4 has shifted into focus as it is the most
common chemokine receptor expressed on cancer cells [16].
It was suggested to play an important role in tumor spread
of colorectal, breast, and oral squamous cell carcinoma as all
of them commonly metastasize to SDF-1α expressing organs
[17–20]. Data obtained from in vitro as well as from murine
in vivo models, analyzing the metastatic ability of CXCR4 in
expressing cancer cells, underlined the key role of CXCR4 for
tumor cell malignancy, as activation of CXCR4 by SDF-1α
induced migration, invasion, and angiogenesis of cancer cells
[21–23].

Therefore, we evaluated the expression of CXCR4 in
renal cancer cell lines and specimens and correlated these
results with the patients’ clinicopathological parameters and
survival.

2. Materials and Methods

2.1. Tissue Samples. Renal cell carcinoma samples were
intraoperatively obtained from 113 patients with renal clear
cell carcinoma who underwent surgery at the Department
of Urology of the University of Mainz. The morphological
classification of the carcinomas was conducted according to
World Health Organization (WHO) specifications. Patients
were followed up on a regular basis depending on the
procedure performed.

2.2. Immunohistochemical Staining. The avidin-biotin-
complex method (LSAB+ System-HRP Kit, Dako
Cytomation, Hamburg, Germany) was used to detect the
protein CXCR4 (anti-CXCR4, dilution 1 : 300; Capralogics
Inc., Mass, USA). Formalin-fixed and paraffin-embedded
tissues were deparaffinized and subsequently microwaved
(600 W, 15 minutes) in citrate buffer (ph 6.0). After
preincubation with hydrogen peroxide (LSAB+ System-HRP
Kit, Dako Cytomation, Hamburg, Germany) and human
AB plasma (Department of Transfusion, University of
Mainz, Mainz, Germany), the primary antibodies were
applied for one hour at room temperature. After incubation
with the secondary antibody (LSAB+ System-HRP Kit,
Dako Cytomation, Hamburg, Germany), the avidin-biotin
complex was added and the enzyme activity was visualized
with diaminobenzidine (LSAB+ System-HRP Kit, Dako
Cytomation, Hamburg, Germany). Counterstaining was
performed with haematoxylin (Roth, Karlsruhe, Germany).
For negative controls only the secondary antibody was
used. A negative control was performed for each sample
(N = 113). For positive controls formalin-fixed and
paraffin-embedded tissue samples of the human spleen were
applied.

2.3. Evaluation of Immunostaining. Immunostaining was
evaluated by three authors independently (T.C. Wehler, C.
Graf, S. Biesterfeld), blinded to patient outcome and all clin-
icopathologic findings. The immunohistochemical staining
was analyzed according to a scoring method as previously
validated and described [17]. The tumors were classified into
four groups based on the homogeneous staining intensity:
0, absent; 1, weak; 2, intermediate; 3, strong staining. In the
case of heterogeneous staining within the same sample, the
respective higher score was chosen, if more than 50% of cells
revealed a higher staining intensity. If expression intensity
was exactly in between two scores, the authors agreed on 0.5
point-steps. If evaluations did not agree, specimens were re-
evaluated and reclassified according to the assessment given
most frequently by the observers.

2.4. Statistics. The correlation of CXCR4 staining intensity
with clinicopathological patterns was assessed with the χ² test
and with the unpaired Student t-test (one/two sided), when
appropriate. Survival rates were visualized applying Kaplan-
Meier curves, and P-values were determined by log-rank test.
P < .05 was considered significant and P < .001 highly
significant in all statistical analyses.

3. Results

3.1. Tumor Characteristics and Patient Profiles. The selected
group of patients represents the typical characteristics of
renal cell carcinoma in industrialized countries.

3.2. Immunohistochemical Staining of CXCR4 in Renal Cell
Carcinoma. The staining of normal human kidney tissue
for CXCR4 revealed a cytoplasmatic expression and in only
few specimens an additional weak membranous location of
CXCR4 (see Figure 1). A nuclear staining of CXCR4
was not observed. In renal cell carcinoma, the respective
expression rate for CXCR4 was 100% (113/113) and varied
from weak (34%), intermediate (42%), to strong (24%).
Negative controls of human renal cancer remained negative
for all tissue samples (N = 113, not shown). Glomeruli
did not reveal any CXCR4 expression and thus served
as internal negative control. As internal positive control,
splenic lymphocytes (strong CXCR4 expression) and tubuli
cells (intermediate CXCR4 expression) were used. Similarly,
inflammatory infiltrates in kidney tissue (data not shown)
depicted a strong CXCR4 expression.

3.3. Relevance of CXCR4 Expression in Renal Cell Carcinoma.
Strong CXCR4 expression significantly correlated with dedif-
ferentiated (P = .0005) and progressed renal cell carcinoma,
indicated by T-status (P = .039; see Table 1). Furthermore,
strong CXCR4 expression revealed a significant association
with low hemoglobin values (P = .039) and a nonsignificant
trend towards increased thrombocytes (P = .089/P = .18,
Normal kidney
Renal clear cell carcinoma

**Figure 1:** The figure depicts CXCR4 expression in healthy kidney and cancer samples. While glomeruli did not depict any CXCR4 expression, tubuli did reveal a medium-strong predominantly cytoplasmic CXCR4 expression. All cancer samples did reveal a cytoplasmatic expression of CXCR4 ranging from weak (34%) to medium (42%) and strong (24%).

resp.). No correlation was seen for age, size, survival, or creatinine values.

**4. Discussion**

The expression of the chemokine receptor CXCR4 has been reported in various epithelial, mesenchymal, and hematopoietic tumors. In several entities, its expression was linked to tumor dissemination and poor prognosis [20, 24, 25]. CXCR4 expression can be increased as a result of intracellular second messengers such as calcium [26] and cyclic AMP [27, 28] by the inactivation of the tumor suppressor gene p53 and overexpression of NFkB [29–31], by cytokines like IL-2, IL-10, or TGF-1β [26, 32] and by growth factors such as VEGF and EGF [33, 34]. In addition, Staller and colleagues could demonstrate that CXCR4 is a hypoxia inducible gene with a HIF-1α binding domain, and that its overexpression in clear-cell renal cell carcinoma is due to a loss-of-function of the von Hippel-Lindau (VHL) tumor suppressor protein, which under normoxic conditions directs HIF-1α to ubiquitin-mediated degradation [35]. Loss of VHL stabilizes HIF-1α leading to increased expression of hypoxia-response genes including VEGFA, CXCR4, its ligand SDF1α, and HIF-1α itself [36, 37]. They also reported a positive correlation between strong CXCR4 expression and poor tumor-specific survival independent of tumor stage and differentiation grade. The latter is in contrast to the results obtained in our study.

We analyzed the expression profile of CXCR4 in a series of human renal cell carcinoma cell lines and 113 patients’ samples for which exact tumor staging and followup data were available and correlated the expression profile with clinicopathological data. The human renal cell carcinoma tumor samples that are analyzed revealed varying intensities of CXCR4 expression ranging from weak to strong, as previously described for pancreatic and colorectal cancer [38]. Interestingly, CXCR4 expression was downregulated in 34% and upregulated in 24% of renal cell carcinoma as compared to original tubuli cells. 42% of cancers revealed the identical expression intensity of CXCR4 as tubuli cells. A cytoplasmatic staining of CXCR4 was observed in all cancers, whereas fewer cases depicted an additional membranous localization of CXCR4. These observations are in line with a recently published study by Zagzag and coworkers [44]. Furthermore, it was reported that CXCR4 surface expression was higher in permanent cell lines than in primary tumor samples [39]. Noteworthy, an inducible translocation of CXCR4 from the cytoplasm to the membrane has been reported previously in [29]. In addition, at least in breast cancer cells, inhibited CXCR4 ubiquitination was described as another mechanism contributing to increased CXCR4 surface levels [40].

In our renal cell carcinoma patients, a strong CXCR4 expression was significantly associated as well with progressed cancer as indicated by the T-status as with dedifferentiation. Our results are furthermore in line with recent reports from our group and others, describing a similar effect of CXCR4 on disease progression in other tumor entities [17, 41]. Hence, our data suggest a relevant influence of CXCR4 on proliferation and differentiation of renal cell carcinoma with regard to the in vivo situation. This hypothesis is strengthened by observations in a murine model, where
Table 1: Patient and tumor characteristics dependent on intensity of CXCR4 expression.

<table>
<thead>
<tr>
<th>CXCR4 expression</th>
<th>Weak</th>
<th>Medium</th>
<th>Strong</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>39 (34%)</td>
<td>47 (42%)</td>
<td>27 (24%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Average age (years)</td>
<td>63.8</td>
<td>66.3</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>36 (42%)</td>
<td>8 (30%)</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>50 (58%)</td>
<td>19 (70%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grading</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>65 (78%)</td>
<td>11 (41%)</td>
<td>P = .0005</td>
<td></td>
</tr>
<tr>
<td>3/4</td>
<td>18 (22%)</td>
<td>15 (59%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>64 (76%)</td>
<td>15 (56%)</td>
<td>P = .039</td>
<td></td>
</tr>
<tr>
<td>3/4</td>
<td>20 (24%)</td>
<td>12 (44%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average size (cm)</td>
<td>5.7</td>
<td>6.0</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Survival (months)</td>
<td>29.7</td>
<td>36.8</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Average creatinin (mg/dl)</td>
<td>1.11</td>
<td>1.07</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Average hemoglobin (g/dl)</td>
<td>14.32</td>
<td>13.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average thrombocytes (/nl)</td>
<td>271</td>
<td>313</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

the metastatic capability of CXCR4-expressing RCC cells strongly correlated with CXCR4 protein level on cancer cells and the SDF-1α expression in the target organs [23]. Therefore, CXCR4-expressing cancer cells are certainly attracted to the typical “homing organs” such as lungs, bone marrow, liver, and lymph-nodes showing a high SDF-1α expression [13, 42]. A pathophysiological relevant fact worthwhile to be mentioned is that endothelial cells coexpress SDF-1α and VCAM-1, thus mediating tumor-cell/endothelial cell attachment. CXCR4 activation by SDF-1α induces β-integrin expression, binding VCAM-1 on endothelial cell [43, 44]. Similar pathophysiological processes must be proposed for renal cell carcinoma dissemination.

Therefore, CXCR4 might be an interesting therapeutic target in a multimodal therapy of renal clear cell carcinoma.

Abbreviations

CXCR4: Chemokine receptor 4
EMEA: European Medicines Agency
FDA: Food and Drug Administration
HIF: Hypoxia induced factor
IL: Interleukin
RCC: Renal cell carcinoma
RTK: Receptor-tyrosine kinases
SDF-1α: Stromal cell derived factor 1α
VHL: Von Hippel Lindau
WHO: World health organization.

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