Research Communication

Immunohistochemical Distinction of Metastases of Renal Cell Carcinoma with Molecular Analysis of Overexpression of the Chemokines CXCR2 and CXCR3 as Independent Positive Prognostic Factors for the Tumorigenesis

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Abstract

Renal cell carcinoma (RCC) represents, on average, over 90% of all malignancies of the kidney that occur in adults in both sexes. Chemokine receptors expression has been found in many kinds of cancer and at tumor metastasis site. We determined CXCR2 and CXCR3 expression in RCC by immunohistochemistry method and analyzed the prognostic value of these markers. Our finding demonstrated that CXCR3 were highly overexpressed in renal cancer tissues compared with those adjacent normal kidney tissues ($P<0.001$). The results showed that high expression of CXCR3 was markedly correlated with metastasis ($P=0.021$) and tumor stage ($P=0.031$). CXCR2 were overexpressed in renal cancer tissues compared with those adjacent normal kidney tissues ($P<0.001$). Our result showed that CXCR2 expression was correlated with high grade ($P=0.024$), advanced stage ($P=0.029$) and metastasis ($P=0.018$). The log-rank test revealed that high CXCR2 and CXCR3 expressions are related to poorer overall survival ($P<0.001$; $P<0.001$). In conclusion, this study indicates the correlation of CXCR3 and CXCR3 with progression of RCC. In addition, high CXCR3 andCXCR2 expressions were correlated with shorter overall survival. © 2016 IUBMB Life, 68(8):629–633, 2016

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Introduction

Renal cell carcinoma (RCC) accounts for up to 3% of all malignancies, that occur in adults in both sexes (1–3). In has been documented that one-third of patients with RCC suffering from metastatic tumor, about 40% of them will eventually result in distant metastasis (1), and also the 5-year survival rate of patients with metastatic RCC has been showed to be less than 10% (4). The prognosis of patients with metastatic RCC is known to be poor, that median survival can be less than 1 year (5), no markers exist to identify metastatic patients.

TABLE 1 *Correlation between clinicopathological features and CXCR3/CXCR2 expressions in RCC patients*

	Number	CXCR3			CXCR2		
Parameter	*45*	*Low (n = 11)*	*High (n = 34)*	P *value of CXCR3*	*Low (n = 9)*	*High (n = 36)*	P *value of CXCR2*
Age (yr)							
<55	20	4	16	0.621	3	17	0.542
≥55	25	7	18		6	19	
Sex							
Male	28	5	23	0.534	4	24	0.41
Female	17	6	11		5	12	
Metastasis							
Yes	24	3	21	0.021	2	22	0.018
No	21	8	13		7	14	
Tumor stage				0.031			0.029
I/II	27	8	19		7	20	
III/IV	18	3	15		2	16	
Tumor grade							
Well differentiated	18	3	15	0.512	2	16	0.024
Moderately differentiated	15	4	11		1	14	
Poorly differentiated	12	4	8		6	6	

Therefore, valuable markers for diagnosis and prognosis can be effective (6,7). Chemokines are involved in many cellular functions, such as induction of cell, proliferation, differentiation, and migration of different cell types (8,9). The chemokine receptors are divided into four subgroups (CXC, CC, CX3C, and C) based on the arrangement of the position of conserved cysteine residues (10).

Chemokines has been revealed to be linked to metastasis of many kinds of tumors. Chemokine receptors expression has been found in many kinds of cancer and at tumor metastasis site (9,11,12). CXCR3 is documented to be a classic 7-transmembrane G-protein coupled CXC chemokine receptor that its expression is occurred on activated T-lymphocytes. CXCR3 is expressed in RCC and it has been found that CXCR3 can be involved in tumor metastasis (13). It has been previously demonstrated that that CXCR3 and its ligands were overexpressed in RCC than in corresponding normal renal tissue samples. Furthermore, the relationship between CXCR3 expression and RCC metastasis has been shown (14–16).

Aberrant expression of CXCR2 has been detected in many tumor types (17–19). Analysis of malignant and benign CXCR2 has been reported to be expressed in all breast cancer tissues compared with only ductal epithelial cells that 50% of them expressed this receptor (20). In addition, differential CXCR2 expression was previously detected in breast cancer cell lines. However, the role of CXCR2 and CXCR3 in patients with RCC needs further investigation. In this study, we investigated the association of CXCR2 and CXCR3 with the clinicopathologic factors of patients with RCC. Also, the prognostic value of these markers was analyzed.

Materials and Methods

A total of 45 tissues of RCC and corresponding normal renal specimens were collected from patients who underwent radical nephrectomy at Tehran between 2008 and 2013. The tissues were snapping frozen in liquid nitrogen and were stored at −80 °C until use. Tumors were graded (I–IV) according to the Fuhrman nuclear grading system. The overall survival of patients was defined as the elapsed time between the operation time and death. The clinicopathological factors of patients were indicated in Table 1

Immunohistochemistry

Four-micrometer-thick sections were used for immunohistochemical staining. The slides were incubated in 3% hydrogen

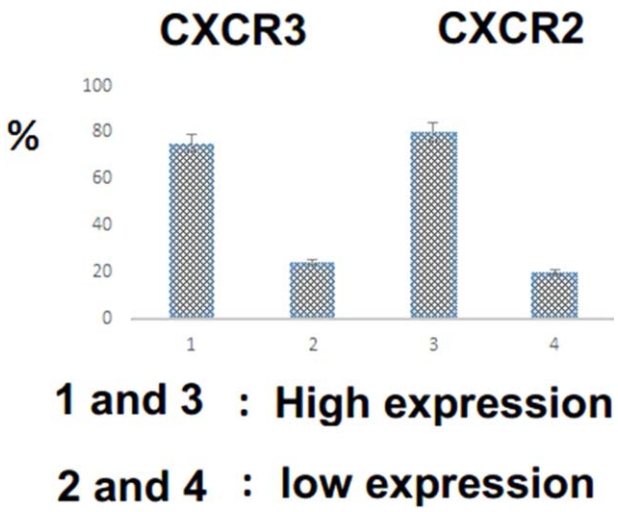

FIG 1 *Low and high expression of CXCR3 and CXCR2 in RCC patient's tissues.*

peroxide for 5 min. The tissue sections immersed in 10 mM citrate buffer for 3 minutes, and then incubated with 10% normal goat serum in PBS for 30 min to block nonspecific binding. After three rinses with PBS buffer, then the sections were incubated with anti-CXCR3 mAbs (1:100 dilution) and anti-CXCR2, overnight at 4 °C. The slides were incubated with a 1:30 dilution biotin-labeled secondary antibodies and streptavidin-peroxidase for 20 min. The brown color indicative of peroxidase activity was developed by incubating with 3,3′diaminobenzidine tetra-hydrochloride. The percentages of tumor cells were as fallow: cell staining was categorized into high-expression groups of CXCR3 and CXCR2 (cell staining of $\geq$30%) and low-expression groups with cell staining of $<$ 30% or no staining groups.

Statistical Analysis

The relationship between expression and clinical factors were analyzed using the χ^2 test or Fisher's exact test. Survival was assessed by Kaplan-Meier method and the log-rank test was applied for analysis of survival curves. Statistical analysis was considered to be statistically significant $P < 0.05$. All statistical analyses were performed with using the SPSS 17.0 (IBM, Chicago, IL).

Results

Immunohistochemical Staining Findings

CXCR3 Expression. Our finding demonstrated that CXCR3 were highly overexpressed in renal cancer tissues compared with those adjacent normal kidney tissues ($P < 0.001$). High level of CXCR3 expression was observed in 34 patients (75.55%) and low expression was found in 11 patients (24.44%) (Fig. 1). The results showed that high expression of CXCR3 was markedly correlated with metastasis ($P = 0.021$) and tumor stage ($P = 0.031$). Furthermore, no correlation was

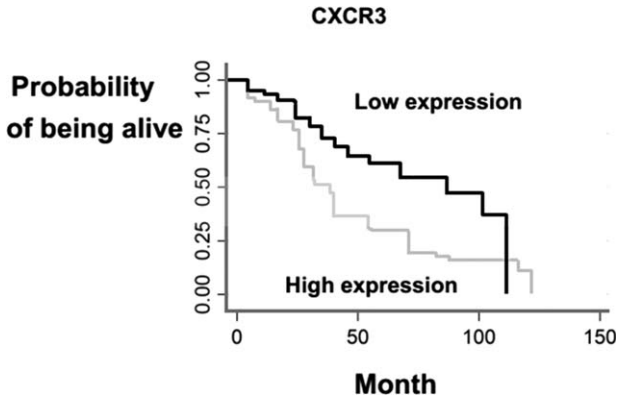

FIG 2 *Survival analysis of RCC patients by Kaplan-Meier method; overall survival rate (CXCR3).*

determined between expression levels of CXCR3 with other clinicopathological characteristics (Table 1).

CXCR 2 Expression. CXCR2 were overexpressed in renal cancer tissues compared with those adjacent normal kidney tissues ($P < 0.001$). High level of CXCR2 expression was observed in 36 cases (80%) and low level of expression was detected in nine cases (20%) (Fig. 1). Our result showed that CXCR2 expression was correlated with high grade ($P = 0.024$), advanced stage ($P = 0.029$), and metastasis ($P = 0.018$). No significant correlation was found between CXCR2 expression in tumor tissues and other clinicopathologic features (Table 1).

The Association of CXCR3 and CXCR2 Expressions with Overall Survival (OS). Figures 2 and 3 show OS rates of patients with RCC in CXCR3 and CXCR2 high- and low-immune expression. There was a statistically significant difference in OS in patients with high and low expression of CXCR3 and CXCR2. Kaplan-Meier survival and log-rank analysis showed that the patients with high CXCR3 had shorter overall survival than those with low expression level ($P < 0.001$). Elevated CXCR2 expression was positively linked to worse overall survival ($P < 0.001$).

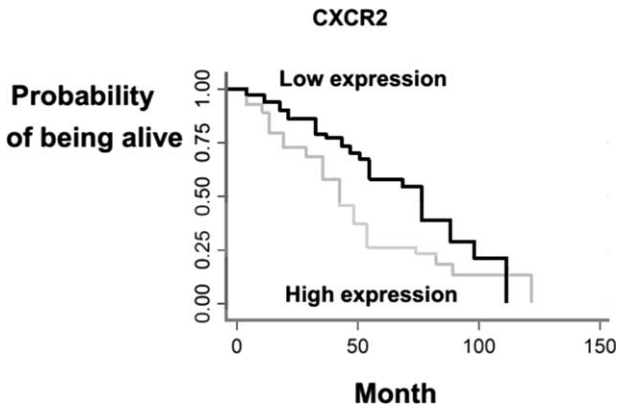

FIG 3 *Survival analysis of RCC patients by Kaplan-Meier method; overall survival rate (CXCR2).*

Discussion

Chemokine receptors expression has been determined in many kinds of cancer and at tumor metastasis site (9,11,12). CXCR3 has been detected to be expressed in RCC and its role in tumor metastasis has been identified (13). Data show that CXCR3 was highly overexpressed in renal cancer tissues compared with those adjacent normal kidney tissues. Furthermore, increased expression of CXCR3 correlates with metastasis and tumor stage. Kaplan-Meier survival and log-rank analysis showed that the patients with high CXCR3 had shorter overall survival than those with low expression level. It has been previously demonstrated that expression of CXCR3 or its ligands is correlated with good prognosis in patients with localized RCC (21,22). On the other hand, CXCR3 is recognized as a poor prognostic factor and promote tumor metastasis (23,24). In agreement with our study, a study indicated that CXCR3 and its ligands were abundant in RCC. They demonstrated the correlation of CXCR3 and CXCR3-A with RCC metastasis (14). Current evidence shows that CXCR3 is the potential candidate for a new therapy target because of its important roles in cancer progression. Furthermore, CXCR3 functions are easily inhibited by the neutralizing CXCR3 antibody. CXCR3 is considered to fulfill the essential conditions of a molecular therapy target (14,25). Regarding the metastasis of patients, CXCR3/CXCL10 interaction are involved in cell migration and invasion (14). Further investigations are necessary to define the pathophysiologic significance of CXCR3 in developing RCC. On the other hand, CXCR2 were overexpressed in renal cancer tissues compared with those adjacent normal kidney tissues. Our findings suggested that CXCR2 expression is related to higher grade, advanced stage, and metastasis. Furthermore, high CXCR2 expression was correlated with poor overall survival.

Experimental evidence indicated that CXCR2 are involved in the progression of RCC. Expression of CXCR2 has been demonstrated in various types of cancer (17–19). Analysis of malignant and benign CXCR2 has been reported to be expressed in all breast cancer tissues compared with only ductal epithelial cells that 50% of them expressed these receptors (20). High CXCR2 expression has been reported recently in endothelial cells of metastatic RCC. Regarding the orthotopic RCC tumors, it has been demonstrated metastatic potential in CXCR2−/− mice (26). Singh et al. showed CXCR2 expression in the neoplastic cells, as well as CXCR2 expression was linked to higher grade, advanced stage, and metastases. Furthermore, CXCR2 significantly affected survival time when univariate analysis has been used. These data are more or less consistent with findings of our study. It has been shown that silencing of CXCR2 gene is correlated with pancreatic tumor growth (27) and arrested cells of ovarian carcinoma at G0/G1and G2/M phases of the cell (28). In addition, CXCR2 was revealed to act as a suppressor of the proapoptotic factors, while are positively associated with increased expression of antiapoptotic proteins (27), thereby assisting neoplastic cells to resist chemotherapy. Further investigations are required to clarify the role CXCR2 expression in RCC.

Conclusions

In conclusion, this study indicates the correlation of CXCR3 and CXCR3 with progression of RCC. In addition, high CXCR3 andCXCR2 expressions were correlated with shorter overall survival.

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