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Original article

High expression of CXC chemokine receptor 6 associates with poor prognosis in patients with clear cell renal cell carcinoma

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Abstract

Purpose: Accumulating evidence indicates that CXC chemokine receptor 6 (CXCR6) has a crucial role in cancer development and progression, however, its role in clear cell renal cell carcinoma (ccRCC) remains obscure. The aim of this study is to investigate the prognostic value of CXCR6 expression in patients with ccRCC following surgery.

Materials and methods: This study retrospectively included 239 patients with ccRCC who underwent nephrectomy and had paraffin tissue available at a single center. CXCR6 expression in tumor tissue was evaluated by immunohistochemistry and its associations with overall survival (OS) and recurrence-free survival (RFS) were investigated.

Results: A total of 47.3% tumors were considered as high expression of CXCR6, which was significantly associated with the male sex ($P = 0.003$) and high Fuhrman grade ($P < 0.001$). A high expression of CXCR6 indicated a reduced OS ($P < 0.001$) and RFS ($P = 0.007$). Multivariate analysis demonstrated that CXCR6 expression was an independent prognostic factor of OS (hazard ratio = 2.604; 95% CI: 1.338–5.068; $P = 0.005$) and RFS (hazard ratio = 1.957; 95% CI: 1.065–3.595; $P = 0.031$). Subgroup analysis found that CXCR6 expression could differentiate survival risks among patients with high-risk disease. Moreover, a nomogram integrating CXCR6 expression and traditional clinical and pathologic features was established and predicted postsurgical recurrence-risk well at 3- and 5-year.

Conclusions: The expression of CXCR6 in tumor tissue may serve as a potential prognostic biomarker to refine clinical prognosis prediction combined with traditional clinical and pathological analysis for patients with ccRCC after surgery.

Keywords: Clear cell renal cell carcinoma; CXC chemokine receptor 6; Prognostic biomarker; Overall survival; Recurrence-free survival

1. Introduction

In 2017, approximately 63,990 new cases and 14,400 deaths are projected to occur from kidney and renal pelvis cancer in the United States of America [1]. In China, the incidence of kidney cancer is rising, with almost 66,800 new cases and 23,400 deaths in 2015 [2]. Clear cell renal cell carcinoma (ccRCC) is the most common type of kidney cancer. Despite advances in early detection, 20% of the patients with RCC after curative nephrectomy will have a local or metastatic relapse during the follow-up [3]. For metastatic RCC, in spite of the development of targeted and immunostimulatory therapies, durable and effective responses remain a challenge. Thus, accurate postoperative

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outcome prediction and diverse treatment options based on individual molecular identification are urgently needed.

CXC chemokine receptor 6 (CXCR6) belongs to CXC chemokine family that is well known to be involved in different steps of tumor progression [4]. The interaction between CXCR6 and its ligand CXCL16 has been reported in regulating cancer invasion and metastasis [5]. Overexpression of CXCR6 is found in many types of human tumors and correlates positively with tumor aggressiveness including prostate cancer [6,7], breast cancer [8,9], lung cancer [10], and cervical cancer [11]. Several investigations have reported that high intratumoral expression of CXCR6 is an adverse prognostic indicator in various human cancer types such as hepatocellular carcinoma [12], prostate cancer [13], cervical cancer [11], and Ewing sarcoma [14]. In a previous study, Gutwein et al. [15] found that intratumoral expression of CXCR6 was not associated with the length of overall survival (OS) in a cohort of only 104 RCC cases consisting of 82 ccRCC, 17 papillary RCC, and 5 chromophobe RCC cases. Owing to the small sample size and ignorance of prognostic differences between ccRCC and other subtypes of RCC in that study, the prognostic significance of CXCR6 expression in RCC remains to be investigated.

In this study, we sought to investigate the associations of intratumoral CXCR6 expression with clinicopathologic characteristics and prognosis in patients with ccRCC. The immunohistochemistry (IHC) was used to assess the expression of CXCR6 in tumor tissue. Furthermore, a prognostic nomogram based on multivariate analysis was established to refine individual postoperative recurrence-risk stratification.

2. Patients and methods

2.1. Patients

This study enrolled 239 patients with ccRCC who underwent nephrectomy at Zhongshan Hospital of Fudan University during the year of 2008. The inclusion criterions were as follows: (1) ccRCC confirmed by histopathology; (2) no history of anticancer therapies before surgery; (3) no history of other malignancies; (4) no bilateral renal cancer at diagnosis; (5) no perioperative mortality; (6) archived paraffin tissue available for study. For each patient, the following clinical and pathologic data were collected: age at surgery, sex, TNM stage [16], Fuhrman grade [17], tumor size, tumor necrosis, and Eastern Cooperative Oncology Group performance status (ECOG-PS) before surgery. Histopathologic reviews on microscopic slides from all tumor specimens were performed by one experienced pathologist to confirm reported pathologic features. Nodal metastasis was determined by inoperative and pathologic findings, and distant metastasis was defined according to radiographic examinations. The UCLA Integrated Scoring System (UISS) classified all the patients into 3 survival-risk levels: low-risk (score 1), intermediate-risk (score 2), and high-risk (score ≥3) based on TNM stage, Fuhrman grade and ECOG-PS [18], and the Leibovich score stratified the localized patients with RCC (T1–3N0M0) into 3 recurrence-risk levels: low-risk (score: 0–2), intermediate-risk (score: 3–5), and high-risk (score ≥ 6) based on T stage, N stage, Fuhrman grade, tumor size, and tumor necrosis [19].

Patients with localized RCC (T1–3N0M0) were treated with radical or partial nephrectomy. Patients with metastatic RCC (N1 or M1) were treated with cytoreductive nephrectomy followed by interferon-alfa-based immunotherapy or tyrosine kinase inhibitors therapy. After surgery, patients were followed up with physical examination, laboratory tests, chest imaging, and abdominal ultrasound or computed tomography every 6 months for the first 2 years and annually thereafter. The clinical endpoints of interest were OS and recurrence-free survival (RFS), which were calculated from the date of nephrectomy to the date of death from all causes and recurrence, respectively, or censored at last follow-up. Follow-up data were updated in March 2014. The median follow-up of this study was 69 months (interquartile range [IQR]: 46–72 mo). There were 45 cases who died with a median of 24 months (IQR: 11–47 mo) after surgery, and 49 cases suffering recurrence with a median of 35 months (IQR: 12–52 mo) after surgery at last follow-up. Among 194 patients still alive at last follow-up, the median duration of follow-up was 70 months (IQR: 68–74 mo). This study was approved by the Zhongshan Hospital's Ethics Committee and informed consent was obtained from each patient.

2.2. Immunohistochemistry

Primary antibody against human CXCR6 (dilution: 1:400; ab8023, Abcam, Cambridge, MA) was applied for IHC staining on tissue microarrays. Tissue microarrays construction and IHC protocol were described previously [20]. The CXCR6 staining on each of tumor specimens was evaluated by 2 independent pathologists blinded to clinical outcome, using a semiquantitative immunoreactivity score (IRS) system which ranged from 0 to 300, by multiplying the staining intensity (0, no staining; 1, weak; 2, moderate; and 3, strong) and staining extent scored as the percentage of positive tumor cells (0%–100%). The median value of IRS (110) was selected as the cutoff point. Thus, IRS more than 110 was considered as high CXCR6 expression.

2.3. Statistical analysis

Pearson χ^2 test and Mann-Whitney U test were used to compare categorical and continuous variables, respectively. Kaplan-Meier analysis with log-rank test was used to compare survival curves. The Cox proportional hazards regression model was applied for univariate and multivariate analysis. Those prognostic factors that had