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CXCR1 expression predicts benefit from tyrosine kinase inhibitors therapy in patients with metastatic renal cell carcinoma

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Abstract

Purpose: CXCR1 signaling promotes tumor progression in various cancers, and clinical trial has proved efficacy of CXCR1 inhibitor in metastatic breast cancer. Therefore, we investigated the prognostic value of CXCR1 in patients with metastatic renal cell carcinoma (mRCC) receiving tyrosine kinase inhibitors (TKIs) therapy.

Materials and Methods: Patients treated with sunitinib or sorafenib were retrospectively enrolled ($n = 111$). CXCR1 expression was assessed by immunohistochemical staining of tissue microarrays of primary tumor, and its association with prognosis and therapeutic response were evaluated. To explore possible mechanism related to CXCR1 expression, gene set enrichment analysis was performed based on The Cancer Genome Atlas cohort.

Results: High CXCR1 expression was associated with poorer overall survival ($P = 0.015$) and was an independent prognostic factor for patients with mRCC treated by TKIs (Hazard Ratio $= 1.683$, 95% Confidence Interval: 1.109–2.553, $P = 0.014$). CXCR1 expression was also associated with worse therapeutic response of TKIs ($P = 0.017$). Thirteen pathways, including hypoxia and angiogenesis, were identified to be enriched in CXCR1 positive patients.

Conclusions: High CXCR1 expression indicates reduced benefit from TKIs therapy in patients with mRCC. The mechanism may be attributed to the enriched pathways of hypoxia and angiogenesis in CXCR1 positive patients. CXCR1 may be a potential therapeutic target for mRCC, but further studies are required.

Keywords: Metastatic renal cell carcinoma; CXCR1; Tyrosine kinase inhibitors; Prognosis; Therapeutic response

1. Introduction

Renal cell carcinoma (RCC) is the sixth and tenth most common cancer in men and women, respectively [1]. Localized RCC has a favorable prognosis after surgical resection, but for 32% of the RCC patients with advanced or metastatic disease, the prognosis is poor [1]. Although tyrosine kinase inhibitors (TKIs) such as sunitinib and sorafenib are recommended for the treatment of advanced or metastatic RCC (mRCC), the response rate is only about 30% [2]. IMDC model was established by Heng et al. [3] to stratify risk group for mRCC patients treated with vascular endothelial growth factor (VEGF) targeted agents. However, this model solely includes clinical factors and did not take into account of tumor heterogeneity or molecular biomarkers that may influence therapeutic response or prognosis.

Cysteine-X-cysteine chemokine receptor type 1 (CXCR1), also known as interleukin-8 (IL-8) receptor alpha, is a G protein-coupled receptor that can be bind and activated by chemokine (C-X-C motif) ligand 6 (CXCL6) and CXCL8 (also

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known as IL-8) [4]. CXCR2 shares 76% sequence homology with CXCR1 and bind to CXCL8 with similar affinity, but unlike CXCR1 that only weakly binds to other ELR+ chemokines, CXCR2 interacts with all other ELR+ chemokines (CXCL1–3, 5–7) with high affinity [5]. The IL-8–CXCR1/2 axis plays a critical role in various cancers, promoting tumor proliferation, invasion, angiogenesis, metastatic potential, drug resistance, and immunosuppression [4,5]. In RCC patients treated with sunitinib, circulating IL-8 level and IL-8 gene rs1126647 polymorphism have been reported to be associated with overall survival [6,7]. IL-8 has also been proved to be an important contributor to sunitinib resistance in clear cell renal cell carcinoma (ccRCC) [8]. As receptor of IL-8, the expression of CXCR1 in human mRCC and its correlation with survival or TKI therapeutic response have not been studied yet.

In the present study, we evaluated CXCR1 expression in primary tumor tissues from mRCC patients treated with sunitinib or sorafenib, and explored its relation with TKI therapeutic response and prognosis. Since the CXCR1/2 inhibitor reparixin (also known as repertaxin) has been proved safe and effective for patients with metastatic breast cancer in phase Ib clinical trial [9], the results of this study may not only shed light upon the prognostic value of CXCR1 in mRCC patients treated with TKIs, but also provide evidence for further clinical trial of CXCR1 inhibitor.

2. Patients and methods

2.1. Patient population

A total of 138 mRCC patients who received sunitinib or sorafenib therapy during March 2005 to June 2014 at Zhongshan Hospital, Fudan University (Shanghai, China) were screened, and 27 were excluded for unavailable clinical data, loss of follow-up, former history of malignancy or targeted therapy, unavailable tissue sample or sample with over 80% necrotic/hemorrhagic area. The research protocol was approved by the clinical research ethics committee of Zhongshan Hospital (NO. B2015-030).

All data were retrospectively collected from medical records databases. Disease progression and response of therapy were defined according to RECIST 1.1 criteria and the last follow-up time was January 2017 [10]. Overall survival (OS) and progression-free survival (PFS) were defined as the time from initiation of TKI therapy to death (of any cause) and to the date of disease progression, respectively. Two pathologist (Yuan Ji and Jun Hou) reconfirmed histological type and nuclear grade of all the patients based on ISUP Vancouver Classification and Fuhrman nuclear grading system [2,11]. Initial TNM stage was classified according to recommendation of EAU guidelines [2]. Risk group was classified according to IMDC model [3].

2.2. Tissue microarray and immunohistochemistry

Two representative circular tumor cores with diameter of 3 mm were selected from each sample to construct tissue microarray. CXCR1 antibody (1:3200 dilution, R&D Systems, Minneapolis, MN) was used in immunohistochemical staining, and the procedure was performed as previously described [12]. Two pathologists, unaware of follow-up data, evaluated CXCR1 expression according to IRS algorithm. The IRS score was calculated by multiplication of staining intensity of tumor cells (0 = negative, 1 = weak, 2 = intermediate, and 3 = strong) and percentage of positive region of tumor cells (1 point for each 10% increment, range 1–10), thus IRS score range from 0 to 30. Both cores of each sample were assessed and mean value of the evaluations was recorded as CXCR1 expression score. CXCR1 expression was dichotomized into high (IRS score $\geq$15) and low (IRS score $<$15) group by Cutoff Finder based on ROC curve [13].

2.3. Gene set enrichment analysis (GSEA)

The public gene expression RNA sequencing dataset of TCGA Kidney Clear Cell Carcinoma cohort (n = 533) was used for GSEA (data downloaded from UCSC Xena https://xenabrowser.net/datapages/). The process was performed by GSEA software 2.0 (Broad Institute, Inc., Cambridge, MA) [14] and pathway identification was based on Hallmark gene sets [15]. Metric for ranking genes was based on Pearson correlation method and analysis was performed 1000 random sample permutations.

2.4. Statistical analysis

Baseline characteristics were analyzed by using Mann–Whitney U test and Pearson χ^2 test. Influence of CXCR1 on OS and PFS was assessed by Kaplan–Meier analysis and log-rank test. Univariate and multivariate Cox proportional hazards regression analyses were performed to identify prognostic factors. For multivariate model, variables with $P < 0.1$ in univariate analysis were selected. Association of CXCR1 expression and therapeutic response was estimated by Kruskal–Wallis H test and Spearman's rank correlation coefficient. All statistical analyses were performed by SPSS Statistics 21.0 (SPSS Inc., Chicago, IL) and two-tailed $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. Baseline characteristics of mRCC patients

CXCR1 expression was evaluated in 111 primary RCC specimens and divided into low (n = 49) and high (n = 62) expression group, with representative immunohistochemical staining photographs shown in Fig. 1A and B. In RCC specimens, CXCR1 expression was mainly detected in tumor cells.

Baseline characteristics of the included patients were demonstrated in Table 1. All patients initially underwent