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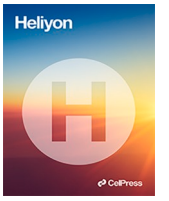

Research article

XCR1: A promising prognostic marker that pinpoints targeted and immune-based therapy in hepatocellular carcinoma

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A R T I C L E I N F O

Keywords:
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A B S T R A C T

Objectives: The lymphotactin receptor X–C motif chemokine receptor 1 (XCR1) is an essential member of the chemokine receptor family and is related to tumor development and progression. Nevertheless, further investigation is required to explore its expression patterns, prognostic values, and functions related to target or immune therapies in patients with hepatocellular carcinoma (HCC).

Materials and methods: The differential expression patterns of *XCR1* and its prognostic influences were performed through The Cancer Genome Atlas (TCGA) and International Cancer Genome Consortium (ICGC) databases. Subsequently, immunohistochemistry (IHC) staining and univariate and multivariate Cox regressions were performed to validate the prognostic values in different subgroups. Furthermore, the potential roles of *XCR1* in predicting target and immune therapeutic responses were also investigated.

Results: Increased expression level of *XCR1* was associated with favorable overall survival (OS) and recurrence-free survival (RFS). Subgroup analysis revealed that a high expression level of *XCR1* or positive immune cell proportion score (iCPS) were associated with favorable OS in the HCC patients with favorable tumor characteristics. In addition, the enhanced *XCR1* expression was associated with the tumor environment scores, immune cell infiltration levels, and the expression levels of immune checkpoint genes. Further analysis revealed that improved expression of *XCR1* was linked to better OS and RFS in HCC patients who received sorafenib.

Conclusion: This study identified that *XCR1* is a valuable prognostic biomarker in the HCC population, especially in those with favorable tumor characteristics. The combination of iCPS status and BCLC status has a synergistic effect on stratifying patients' OS and RFS. Further analyses

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showed that *XCR1* has the potential ability to predict treatment responses to sorafenib and immune-based therapies.

1. Introduction

Hepatocellular carcinoma (HCC) is a major liver malignancy. It is ranked sixth regarding incidence cases and fourth in terms of cancer-related deaths worldwide [1]. Viral hepatitis, alcohol consumption, and non-alcoholic fatty liver disease are the most common risk factors for HCC pathogenesis [2]. Despite the remarkable advancements in diagnosis, surgical treatment, interventional intervention, and comprehensive therapies, the prognosis of HCC patients is still unsatisfactory due to the postoperative recurrence and metastasis [3]. Hence, exploring novel prognostic biomarkers and detailed pathogenesis of HCC, especially in early-stage HCC, is essential to identify survival assessment and develop effective treatment regimens.

The lymphotactin receptor X–C motif chemokine receptor 1 (XCR1) is an essential member of the chemokine receptor family. It is the only chemokine receptor selectively expressed in "Batf3-IRF-8-Id2-dependent dendritic cells (DCs)" or as "CD8 alpha (+) conventional DCs" that is potent in antigen presentation to T cells and facilitates cytotoxic T-cell response [4,5]. Emerging evidence indicates that XCR1 binds with its ligand, X–C Motif Chemokine Ligand 1 (XCL1), which is closely related to an organism's immunological function [6,7].

Meanwhile, the XCL1/XCR1 axis also contributes to the progression of various malignant diseases, including breast cancer, non-small cell lung cancer and renal cell carcinoma (RCC), etc. [5]. A previous study demonstrated that *XCR1* stimulates the migration of MDA-MB-231 triple-negative breast cancer cells, and the XCL1-XCR1 interaction and its associated signaling molecules were the potential targets for inhibiting migration and metastasis of breast cancer cells [8]. High expression of *XCR1* is related to prolonged overall survival (OS) in patients with clear cell RCC, and knockdown of *XCR1* significantly increased RCC cell proliferation and migration and decreased apoptosis [9,10]. In the HCC cell line, silencing *XCR1* promotes cell migration and invasion *in vitro* [11]. Nevertheless, further investigation is required to determine the prognostic significance of *XCR1* and its potential roles in target and immunological treatments for patients with HCC.

In this study, we analyzed differential expression levels of *XCR1* between the tumor and normal liver tissues and their influences on the patients' survival in different subgroups through public databases. Then, we performed immunohistochemistry (IHC) staining and univariate and multivariate Cox regressions to validate the prognostic values in various subgroups. In addition, the potential values of *XCR1* in the prediction of target and immune therapeutic responses were also investigated. This study demonstrated the prognostic importance of *XCR1* and its potential significance in target and immune therapies.

2. Materials and methods

2.1. Data collection and download

RNA sequencing data, somatic mutation data, clinicopathological characteristics, and survival information for patients with HCC were acquired from The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/) database. The recurrence-free survival (RFS) data of HCC patients were downloaded from the UCSC Xena database (http://xena.ucsc.edu/). In addition, RNA sequencing profiles and survival data were also downloaded from the International Cancer Genome Consortium (ICGC) (https://dcc.icgc.org/) dataset. RNA sequencing matrices were normalized for subsequent analysis. Patients with missing data were excluded.

2.2. Expression patterns and prognostic analyses

Differentially expression analysis was performed between the normal and tumor tissues in TCGA and ICGC databases. To establish the correlation between *XCR1* expression and patient prognosis, survival analysis was conducted and validated in the TCGA and ICGC cohorts, respectively. In addition, a correlation between the *XCR1* expression level and clinical characteristics was also investigated.

2.3. Tissues array chip and reagents

A total of 277 patients with a pathological diagnosis of HCC who underwent hepatectomy from May 2008 to November 2008 in Zhongshan Hospital, Fudan University, were enrolled. It has been verified that none of the patients underwent anti-tumoral therapies before surgery. The current study was conducted in accordance with the Declaration of Helsinki. The protocol of this study was approved by the Institutional Review Board (IRB) of Zhongshan Hospital, Fudan University, and written informed consent was waived by IRB.

The tissue array chip containing 277 HCC tissue samples was obtained. Patient characteristics, including baseline characteristics, preoperative laboratory examinations, and individualized tumor features, were also recorded from the medical records. Survival data, including OS and RFS, was also obtained. The definitions of OS and RFS were according to the previous study [12]. XCR1 antibody (Cell Signaling Technology, D2F8T, Rabbit IgG) was purchased from the Shanghai Universal Biotech Co., Ltd. (Shanghai, China).

2.4. IHC staining and scoring

277 HCC tumor tissues were incubated with the XCR1 antibody by IHC in a tissue array chip. The IHC procedure was according to the manufacturer's instructions. Referring to the cut-off value of PD-L1 staining [13,14], samples with immune cell proportion score (iCPS) ≥1 % were defined as positive staining, otherwise as negative staining. The definition of iCPS was the counts of the strong

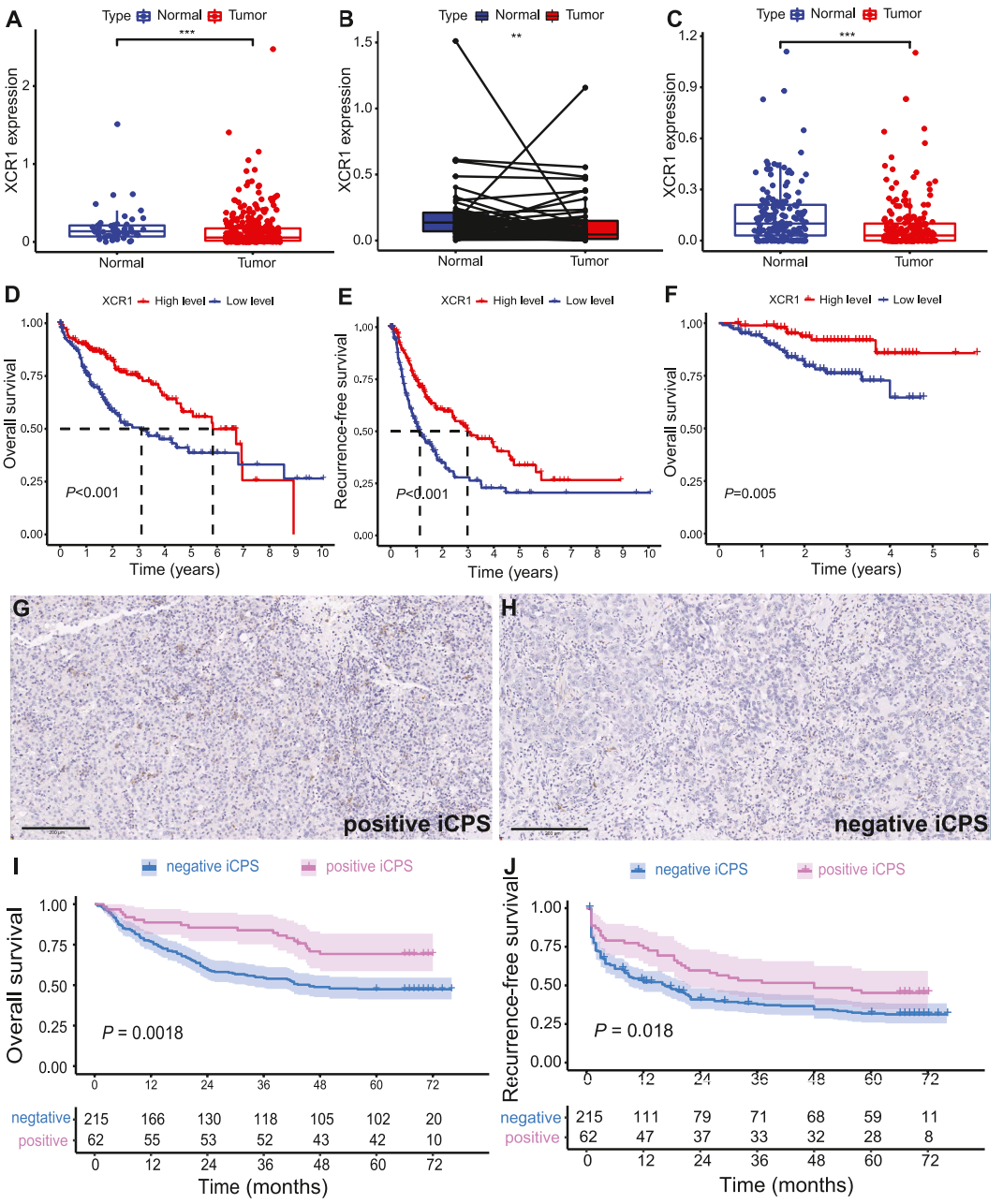

Fig. 1. The expression patterns and prognostic values of *XCR1* in HCC. (A)and (B) The expression levels of *XCR1* in HCC tumor tissues and normal liver tissues in the TCGA dataset. (C) The expression levels of *XCR1* in HCC tumor tissues and normal liver tissues in the ICGC dataset. (D) Kaplan-Meier curve of overall survival in the TCGA dataset. (E) Kaplan-Meier curve of recurrence-free survival in the TCGA dataset. (F) Kaplan-Meier curve of overall survival in the ICGC dataset. The representative images of positive iCPS (G) and negative iCPS (H). (I) Kaplan-Meier curve of overall survival based on the iCPS status in an independent cohort. (J) Kaplan-Meier curve of recurrence-free survival based on the iCPS status in an independent cohort. XCR1, the lymphotactin receptor X–C motif chemokine receptor 1; TCGA, The Cancer Genome Atlas; ICGC, International Cancer Genome Consortium; iCPS, immune cell proportion score. ***, $P < 0.001$; **, $P < 0.01$.

intensity of XCR1 (brown staining) of tumor-associated immune cells/the total counts of tumor-associated immune cells. All IHC slides were scanned and viewed through Aperio ImageScope software. The counts of positive cells and iCPS were randomly counted in the ten fields of view (a magnificent level at 20× in Aperio ImageScope) and quantified by the two independent and experienced pathologists in a double-blind manner.

2.5. Tumor microenvironment analysis

The infiltration levels of stromal cells and immune cells were calculated through the R package of "estimate" [15] and shown by StromalScore, ImmuneScore, and ESTIMATEScore. Then, the differential infiltration levels of 22 immune cells between the high and low *XCR1* expression groups were compared using the "ssGSEA" method. Correlation analysis was further conducted to explore the relationship between *XCR1* and 34 immune checkpoint genes' expression levels. Then, immunophenoscore (IPS) was investigated by the Cancer Immunome Database (TCIA, https://tcia.at/home). IPS was composed of the expression of major histocompatibility complex (MHC) molecules, effector cells, immunomodulators, and suppressor cells, and it was associated with immunogenicity levels [16].

2.6. Correlation between single-nucleotide variant and XCR1

The tumor mutational burden (TMB) in HCC samples was calculated by counting the total non-synonymous mutations using the data from the TCGA database. The correlation between *XCR1* expression and TMB was determined by the Spearman method. Prognostic differences were investigated among four groups: the high-TMB + high-*XCR1* (H-TMB + H-*XCR1*) group, the high-TMB + low-*XCR1* (H-TMB + L-*XCR1*) group, the low-TMB + high-*XCR1* (L-TMB + H-*XCR1*) group, and the low-TMB + low-*XCR1* (L-TMB + L-*XCR1*) group.

2.7. Statistical analysis

Continuous variables were compared by *t*-test. Categorical variables were assessed by the Chi-square or Fisher exact test appropriately. Kaplan-Meier curves were drawn to investigate the survival differences, and log-rank tests were performed in the survival analyses. The median expression value of *XCR1* was used as a cut-off value in the Cox regression analysis. The median TMB value was applied as a threshold to screen the high- and low-TMB patients. Furthermore, the prognostic values of *XCR1* in HCC patients treated with sorafenib were analyzed using the online dataset of Kaplan-Meier plots available at http://kmplot.com/analysis/ [17]. All data analysis was conducted using R (version 3.6.1, Lucent Technologies, USA, https://www.r-project.org) and Strawberry Perl (version 5.32.0.1, https://www.perl.org). A two-tailed *P*-value <0.05 indicates a statistically significant difference.

3. Results

3.1. High expression of XCR1 was associated with favorable survival

Differentially expression analysis revealed that the *XCR1* expression level was decreased in the tumor tissues compared to the normal tissues in the TCGA cohort (Fig. 1A and B). A similar expression pattern was identified in the ICGC dataset (Fig. 1C). High *XCR1* expression in the tumor tissues was found to be significantly associated with improved OS ($P < 0.001$) (Fig. 1D), as well as favorable RFS ($P < 0.001$) (Fig. 1E), according to the Kaplan-Meier survival analysis. An independent dataset validated the similar prognostic values of *XCR1* in terms of patient OS ($P = 0.005$) (Fig. 1F). These findings suggested that *XCR1* may serve as a potential prognostic indicator in HCC patients.

In addition, we assessed the XCR1 protein expression levels in the 277 HCC patients by IHC staining. Our findings revealed that iCPS was present in 22.4 % of the 277 HCC tissue samples analyzed. The representative images of positive and negative patients were displayed in Fig. 1G and H. To validate the prognostic values of XCR1, Kaplan-Meier curves were drawn to compare the OS and RFS based on the scored iCPS. Consequently, the patients with positive iCPS had significantly improved OS and RFS compared to those with negative iCPS (OS, $P = 0.0018$; RFS, $P = 0.018$) (Fig. 1I and J). These findings further validate that the expression levels of *XCR1* could be valuable in predicting the survival of patients with HCC.

3.2. Correlation between expression levels of XCR1 and clinical characteristics

To understand the relationship between *XCR1* and various clinical characteristics in HCC patients, we analyzed differences in clinical characteristics between the high and low *XCR1* expression groups based on the TCGA dataset (Supplementary Figs. S2A–C). Consequently, the expression level of *XCR1* was found to have a strong correlation with both the tumor stage and T stage in patients with HCC (Supplementary Fig. S2A). Higher *XCR1* expression was observed in the patients with stage I rather than stage III ($P = 0.0031$, Supplementary Fig. S2B). No differences were found between the patients with stage I and stage IV ($P = 0.210$). Moreover, higher *XCR1* expression was observed in the patients with T1 than those with T2 or T3 ($P = 0.043$, $P = 0.001$, respectively, Supplementary Fig. S2C). We also compared the differences of baseline characteristics between the iCPS positive and negative groups. Higher proportions of patients with BCLC 0 stage, smaller tumor size, absent of MVI were observed in the iCPS positive group than those in the negative group (Supplementary Table S1). These results suggested that high expression levels of *XCR1* are associated with

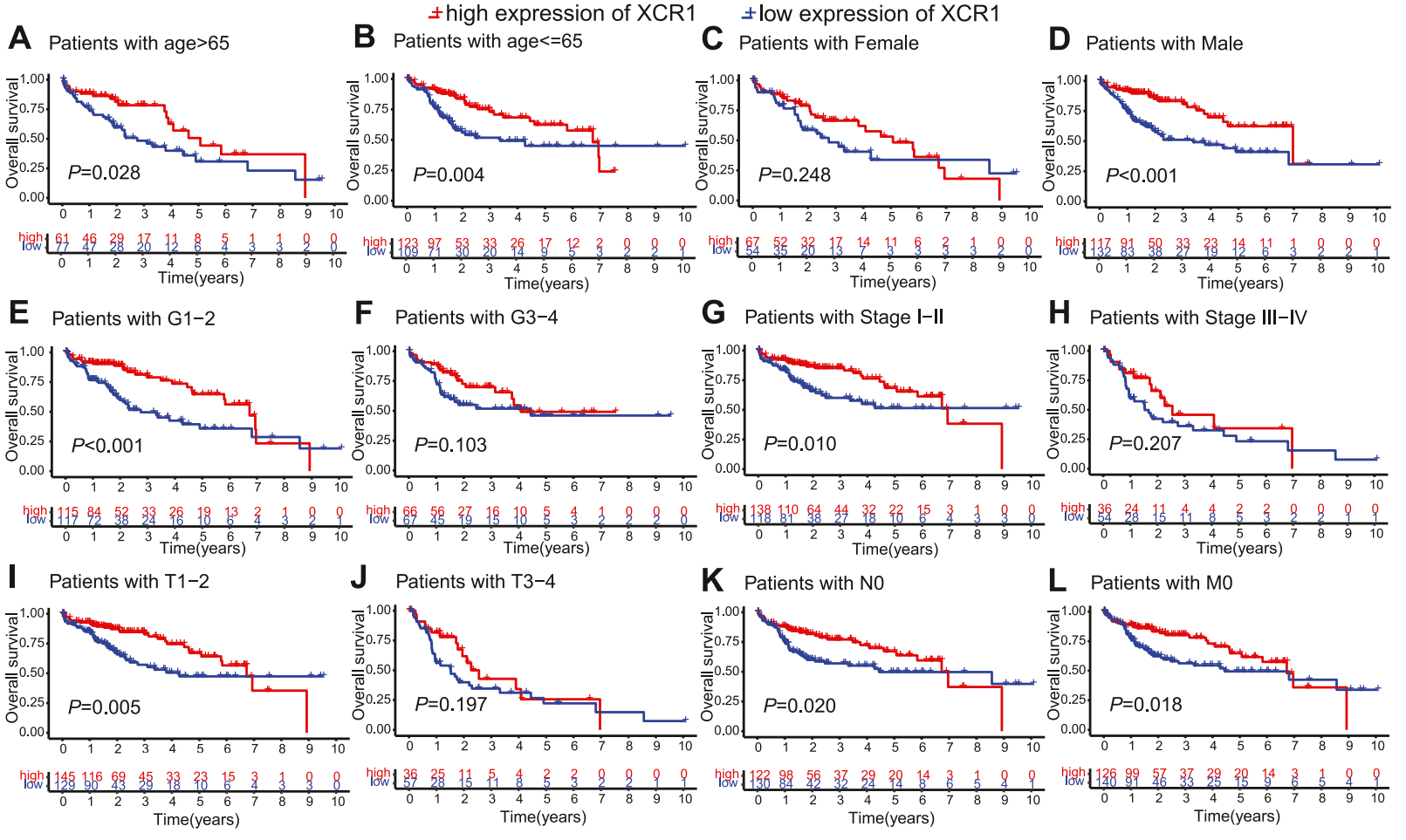

Fig. 2. Kaplan-Meier curve of overall survival based on the expression levels of *XCR1* in the TCGA dataset. Survival curves of overall survival among patients age >65 (A), patients age≤65 (B), female patients (C), male patients (D), patients with G1-2 (E), patients with G3-4 (F), patients with stage I-II (G), patients with stage III-IV (H), patients with T1-2 (I), patients with T3-4 (J), patients with N0 (K), and patients with M0 (L). The median expression value of *XCR1* was used as a threshold. XCR1, the lymphotactin receptor X–C motif chemokine receptor 1; TCGA, The Cancer Genome Atlas.

earlier stage and favorable tumor characteristics in patients with HCC.

3.3. Subgroup analysis identified the roles of XCR1 in patients with favorable tumor characteristics

We further performed the subgroup analysis to analyze the prognostic values of *XCR1* in different populations. Notably, OS was significantly stratified based on *XCR1* expression levels in patients aged >65 years ($P = 0.028$, Fig. 2A) and ≤65 years ($P = 0.004$,

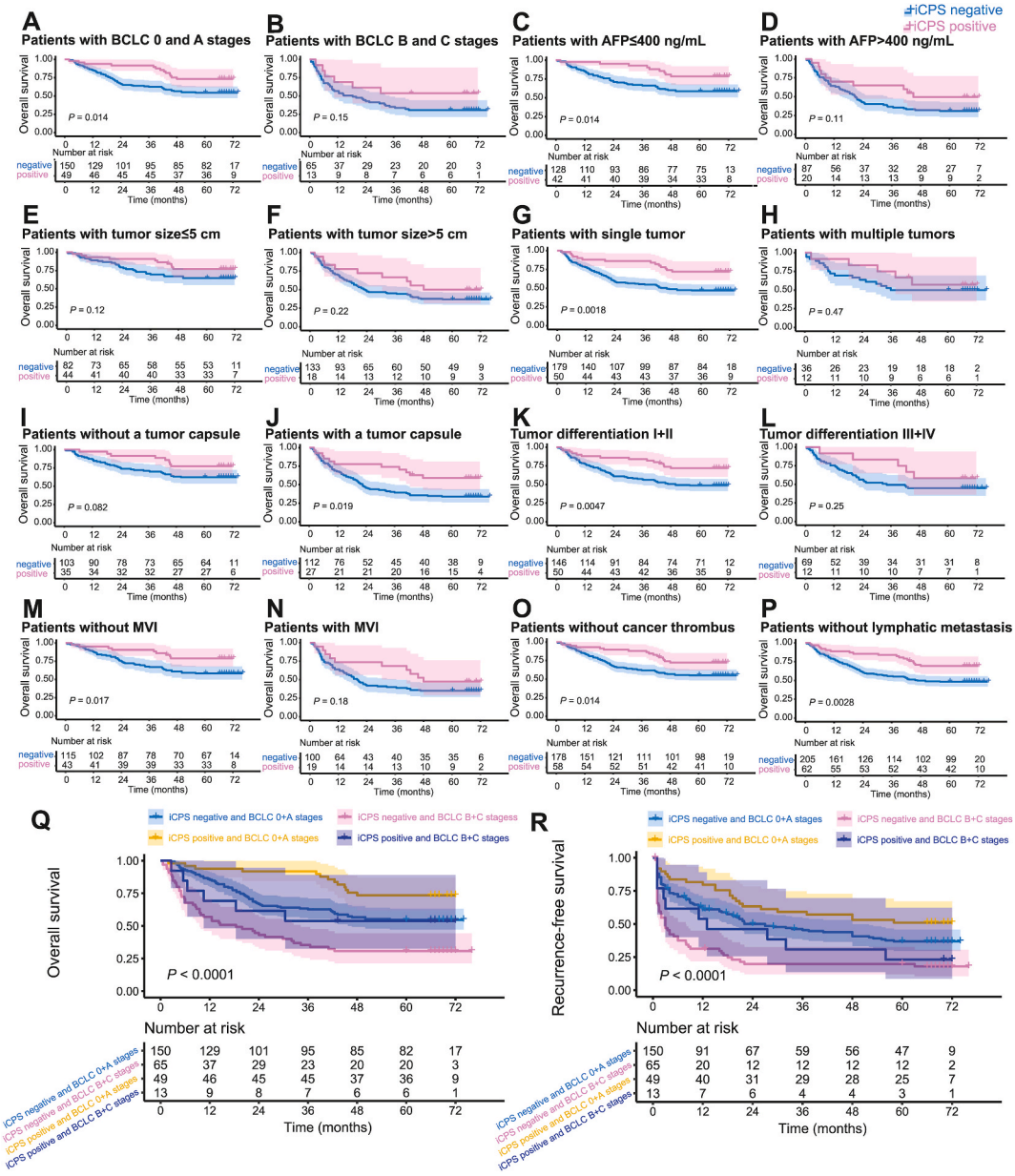

Fig. 3. Kaplan-Meier curve of overall survival based on the iCPS status in an independent cohort. Survival curves of overall survival among the patients with BCLC 0 and A stage (A), patients with BCLC B and C stage (B), patients with AFP ≤400 ng/mL (C), patients with AFP >400 ng/mL (D), patients with tumor size ≤5 cm (E), patients with tumor size >5 cm (F), patients with single tumor (G), patients with multiple tumors (H), patients without a tumor capsule (I), patients with a tumor capsule (J), patients with tumor differentiation I + II (K), patients with tumor differentiation III + IV (L), patients without MVI (M), patients with MVI (N), patients without cancer thrombus (O), and patients without lymphatic metastasis (P). (Q) Kaplan-Meier curve of overall survival integrating the iCPS status with BCLC stage in an independent cohort. (R) Kaplan-Meier curve of recurrence-free survival integrating the iCPS status with BCLC stage in an independent cohort. Samples were considered positive if they had an immune cell proportion score (iCPS) of 1 % or above. Samples with an iCPS below 1 % were considered negative. iCPS, immune cell proportion score; BCLC, Barcelona Clinic Liver Cancer; AFP, alpha-fetoprotein; MVI, microvascular invasion.

Fig. 2B), male patients ($P < 0.001$, Fig. 2D), patients with pathological grade 1–2 ($P < 0.001$, Fig. 2E), patients with American Joint Committee on Cancer (AJCC) stage I–II ($P = 0.010$, Fig. 2G), patients with T stage 1–2 ($P = 0.005$, Fig. 2I), patients with N0 ($P = 0.020$, Fig. 2K), and patients with M0 ($P = 0.018$, Fig. 2L). However, no survival differences were observed in the female patients ($P = 0.248$, Fig. 2C), patients with grade 1–2 ($P = 0.103$, Fig. 2F), patients with AJCC stage I–II ($P = 0.207$, Fig. 2H), patients with T stage 3–4 ($P = 0.197$, Fig. 2J). These results show that *XCR1* is a promising prognostic factor, especially for patients with favorable tumor characteristics.

Furthermore, we also detected and identified the prognostic roles of XCR1 based on iCPS status in an independent cohort. Survival analysis showed that iCPS positive was related to favorable OS in the patients with Barcelona Clinic Liver Cancer (BCLC) 0 + A stages ($P = 0.014$, Fig. 3A), patients with alpha-fetoprotein (AFP) ≤ 400 ng/mL ($P = 0.014$, Fig. 3C), patients with single tumor ($P = 0.0018$, Fig. 3G), patients with a tumor capsule ($P = 0.019$, Fig. 3J), patients with tumor differentiation I + II ($P = 0.0047$, Fig. 3K), patients without MVI ($P = 0.017$, Fig. 3M), patients without cancer thrombus ($P = 0.014$, Fig. 3O), and patients without lymphatic metastasis ($P = 0.0028$, Fig. 3P). However, no differences were observed in the patients with BCLC B + C stages ($P = 0.150$, Fig. 3B), patients with AFP >400 ng/mL ($P = 0.110$, Fig. 3D), patients with tumor size ≤5 cm ($P = 0.120$, Fig. 3E), patients with tumor size >5 cm ($P = 0.220$, Fig. 3F), patients with multiple tumors ($p = 0.470$, Fig. 3H), patients without a tumor capsule ($P = 0.082$, Fig. 3I), patients with tumor differentiation III + IV ($P = 0.250$, Fig. 3L), and patients with microvascular invasion (MVI) ($P = 0.180$, Fig. 3N). For RFS, similar tendencies but no statistical differences were observed in the mentioned subgroups (Supplementary Fig. S2). These findings suggested that *XCR1* was a hub gene related to the OS of HCC patients with BCLC 0 + A stages.

3.4. Univariate and multivariate Cox regression in HCC patients with BCLC 0 + A stages

We also detected the significant prognostic variables in patients with BCLC 0 + A stages based on the clinical information and IHC staining results. Univariate Cox regression analysis demonstrated that AFP >400 ng/mL (2.318 [1.495–3.594], $P < 0.001$), tumor size >3 cm (2.872 [1.799–4.585], $P < 0.001$), patients with microvascular invasion (1.756 [1.129–2.732], $P = 0.013$), and tumor without capsule (2.229 [1.433–3.469], $P < 0.001$) were associated with worse OS, while iCPS positive was correlated to the better OS (0.482 [0.266–0.872], $P = 0.016$) (Table 1). Multivariate Cox regression analysis revealed that AFP >400 ng/mL (2.059 [1.319–3.213], $P = 0.001$) and tumors without capsule (1.958 [1.238–3.096], $P = 0.004$) were significant risk factors associated with worse OS, while iCPS positive was a protective parameter correlated to better OS (0.523 [0.286–0.955], $P = 0.035$) (Table 1). These findings further confirmed that *XCR1* is an independent risk factor associated with OS in patients with early-stage (BCLC stage 0 and A).

We further analyzed survival differences by integrating iCPS status with BCLC status. Intriguingly, patients with iCPS positive and BCLC 0 + A stages showed favorable OS and RFS (Fig. 4Q and R), while patients with iCPS negative and BCLC B + C stages displayed dismal OS and RFS (Fig. 4Q and R). These findings suggested that the combination of iCPS status and BCLC status has a synergistic effect on the stratification of patients' OS and RFS.

3.5. Correlation between XCR1 and tumor microenvironment or TMB

As the only chemokine receptor selectively expressed in the cDCs, differential expression levels of *XCR1* could be associated with the extent of immune cell infiltration. According to the results of the analysis of the estimation algorithm, StromalScore, ImmuneScore, and ESTIMATEScore were significantly higher in the high *XCR1* expression group compared to the low *XCR1* expression group

Table 1
Univariate and multivariate Cox regression analysis based on different clinical characteristics and OS in patients with HCC.

Variables	Univariable model		Multivariable model	
	HR (95 % CI)	*P*	HR (95 % CI)	*P*
Age >60 years	0.925 (0.580–1.475)	0.743		
Male	1.279 (0.719–2.276)	0.402		
HBV	1.195 (0.682–2.093)	0.534		
TB > 21 μmol/L	1.322 (0.575–3.036)	0.511		
PT > 14 s	1.101 (0.479–2.529)	0.821		
ALT >40 U/L	1.515 (0.979–2.345)	0.062		
ALB <35 g/L	1.557 (0.888–2.729)	0.122		
GGT >50 U/L	1.593 (0.994–2.553)	0.053		
AFP >400 ng/mL	**2.318 (1.495–3.594)**	**<0.001**	**2.059 (1.319–3.213)**	**0.001**
Tumor size >3 cm	**2.872 (1.799–4.585)**	**<0.001**	1.459 (0.819–2.598)	0.200
Multiple tumors	1.370 (0.432–4.345)	0.593		
Cirrhosis	1.734 (0.918–3.276)	0.090		
MVI	**1.756 (1.129–2.732)**	**0.013**	1.377 (0.869–2.180)	0.173
Without capsule	**2.229 (1.433–3.469)**	**<0.001**	**1.958 (1.238–3.096)**	**0.004**
Differentiation III-IV	1.293 (0.807–2.072)	0.286		
iCPS positive	**0.482 (0.266–0.872)**	**0.016**	**0.523 (0.286–0.955)**	**0.035**

OS, overall survival; HCC, hepatocellular carcinoma; HR, hazard ratio; CI, confidence interval; HBV, hepatitis B virus; TB, total bilirubin; PT, prothrombin time; ALT, alanine aminotransferase; ALB, albumin; GGT, glutamyl transpeptidase; AFP, alpha-fetoprotein; MVI, microvascular invasion; iCPS, immune cell proportion score.

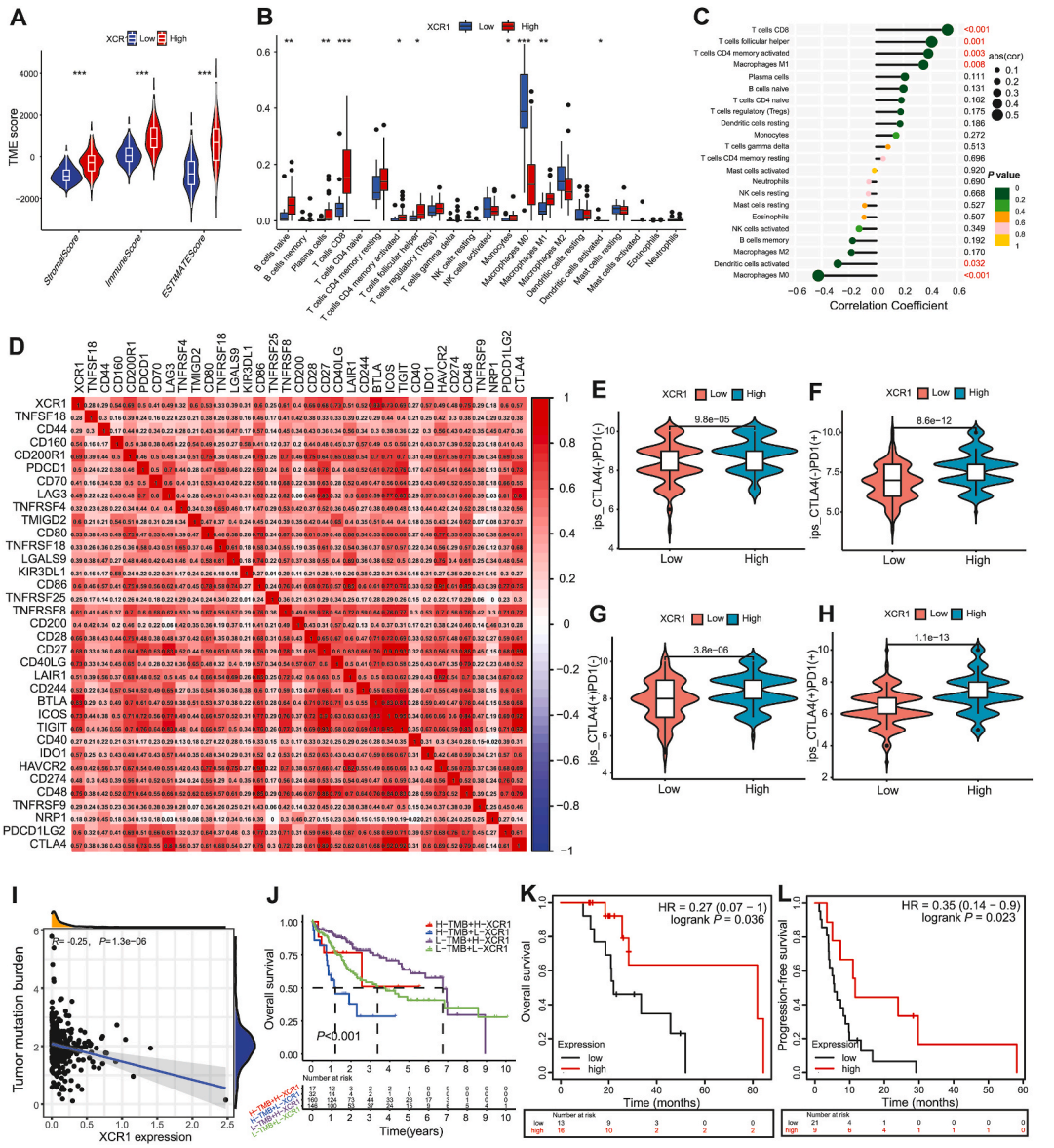

Fig. 4. Tumor environment analysis and prediction values of *XCR1* in target and immune therapies. (A) The distribute levels of StromalScore, ImmuneScore, and ESTIMATEScore between the high- and low-*XCR1* expression groups. (B) The differences in expression levels of 22 immune cells between the high- and low-expression of *XCR1* groups. (C) Correlation between the expression levels of *XCR1* and 22 immune cells. (D) Correlation between the expression levels of *XCR1* and 34 immune checkpoint genes. The differences of immunophenoscore between the high- and low-expression of *XCR1* in the CTLA4 (−) + PD1 (−) group (E), the CTLA4 (−) + PD1 (+) group (F), CTLA4 (+) + PD1 (−) group (G), CTLA4 (+) + PD1 (+) group (H). (I) Correlation between the expression levels of *XCR1* and tumor mutation burden. (J) Kaplan-Meier curves of overall survival for HCC patients with different *XCR1* and tumor mutation burden levels. (K) Kaplan-Meier curve of overall survival based on the different *XCR1* expression levels in the patients who received sorafenib. (L) Kaplan-Meier curve of progression-free survival based on the different *XCR1* expression levels in the patients who received sorafenib. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

(Fig. 4A). The expression level of *XCR1* was significantly positively correlated with the infiltration levels of CD8 T cells, follicular helper T cells, CD4 memory activated T cells and M1 macrophages. However, it negatively correlated with activated dendritic cells and M0 macrophages (Fig. 4B and C).

The correlation analysis between the expression levels of *XCR1* and 34 immunological checkpoint genes revealed a positive correlation (Fig. 4D). The IPS data of HCC patients from the TCIA database showed that the group with high *XCR1* expression had a higher IPS compared to the group with low *XCR1* expression, suggesting that the high *XCR1* group had a better response to immune checkpoint inhibitors (Fig. 4E–H). These findings suggest that *XCR1* could be a valuable indicator for predicting immune therapeutic responses.

TMB is an effective predictor for tumor immunotherapy. The correlation analysis between *XCR1* expression level and TMB revealed a negative correlation in HCC (Fig. 4I). Considering the role of TMB in patient prognosis, we further compared the prognostic value of *XCR1* in patients with high or low TMB scores (Fig. 4J). Intriguingly, the OS of patients in the H-TMB + H-*XCR1* group was superior to that in the H-TMB + L-*XCR1* group, and the OS of patients in the L-TMB + H-*XCR1* was better than that in the L-TMB + L-*XCR1* group. These findings suggested that *XCR1* could stratify patient survival in the high or low TMB subgroups.

3.6. Prognostic analysis of XCR1 in patients who received sorafenib

We also investigated the prognostic values of *XCR1* using the Kaplan-Meier plots in HCC patients who received sorafenib. The optimal cutoff values of *XCR1* were used in this step. Kaplan-Meier curves indicated that better OS (HR = 0.27 [0.07–1], log-rank $P = 0.036$) and PFS (HR = 0.35 [0.14–0.9], log-rank $P = 0.023$) were observed in patients with high *XCR1* expression compared to those with low *XCR1* expression (Fig. 4K and L). These findings suggest that *XCR1* could be a promising predictor for assessing the treatment responses of sorafenib.

4. Discussion

In this study, higher expression of *XCR1* was observed in the tumor tissues than in normal tissues. Furthermore, the enhanced expression of *XCR1* was associated with a favorable pathological stage, contributing to the prolonged RFS and OS. Subgroup analysis demonstrated that a high expression level of *XCR1* or iCPS positive was associated with favorable OS in HCC patients with favorable tumor characteristics. In addition, improved *XCR1* expression was correlated to the tumor environment scores, immune cell infiltration, and the expression levels of immune checkpoint genes, suggesting the potential significance of *XCR1* in the prediction of treatment responses of immunotherapy. Kaplan-Meier curves indicated that high expression of *XCR1* was associated with better OS and RFS in HCC patients who received sorafenib. To the best of our knowledge, no study has comprehensively explored the prognostic significance of *XCR1* in HCC patients with BCLC 0 + A stages and its ability to predict target or immunological treatment responses.

As an essential member of the chemokine receptor family, XCR1 is expressed on both lymphoid and peripheral cross-presenting DC in mice and humans [18–20]. Previous studies have demonstrated that the XCR1/XCL axis has a role in the proliferation, adhesion, migration, and invasion of cancer cell lines and the progression of various cancer types [5,10,11]. Wang et al. reported that silencing *XCR1* promoted migration and invasion of the HCC cell line while overexpressing *XCR1* inhibited promoted migration and invasion of the HCC cell line *in vitro*. The potential molecular mechanism involves the inhibition of Epithelial-Mesenchymal Transition (EMT) [11]. Herein, we further detected the prognostic significance of *XCR1* in the HCC patients. Consequently, a higher expression level of *XCR1* was associated with favorable OS and RFS in patients with HCC. Moreover, similar tendencies of OS and RFS were also identified based on the IHC scoring and survival analysis.

Hepatectomy is the first recommended choice for HCC patients with BCLC 0 + A stages [21]. However, the prognosis after radical hepatectomy is still unsatisfactory. There is an urgent need for the risk assessment of patient survival after surgery [16,22]. Intriguingly, subgroup analyses demonstrated that a higher expression level of *XCR1* was associated with better OS in HCC patients with BCLC 0 + A stages. Multivariate Cox regression identified that *XCR1* was an independent risk factor related to OS in the patients with BCLA 0 + A stages. Moreover, we conducted an in-depth survival analysis by combining iCPS and BCLC status. Interestingly, patients with iCPS positive and BCLC 0 + A stages showed more favorable OS than patients with iCPS negative and BCLC 0 + A stages, while patients with iCPS negative and BCLC B + C stages displayed more dismal OS and RFS than those with iCPS positive and BCLC B + C stages. These findings suggested that *XCR1* is a diagnostic biomarker and a prognostic indicator in HCC patients with favorable tumor characteristics. The combination of iCPS status and BCLC status has a synergistic effect on stratifying patients' OS and RFS.

The immune microenvironment, composed of tumor cells that interact with various immune cells and other cells, has a crucial role in the development and progression of HCC [23,24]. Immune-based therapies such as immune checkpoint inhibitors have revolutionized the systemic treatment of various cancer types [25]. According to bibliometric analysis, dual immunotherapy has become a research hotspot [26]. Inflamed and non-inflamed tumor environment classes of HCC and genomic signatures have been associated with response to immune-checkpoint inhibitors, while low response rate and acquired resistance restricted the treatment efficacy [27,28]. Exploration of an indicator related to immunotherapy responses is significant to selecting low-response-rate patients so they may get survival benefits by promoting antitumor immunity with new nanomaterials [29]. Previous studies have reported that *XCR1*$^+$ DCs are significant in immune therapy [4,30]. Therefore, the expression level of *XCR1* could be an indicator of immunotherapy. Herein, we compared the immune cell infiltration levels and observed a strong correlation between the expression of *XCR1* and various immune cell infiltration. Moreover, the expression level of *XCR1* was significantly positively correlated with the gene expression levels of 32 immune checkpoint genes, which was associated with the formation of an immunotherapy-sensitive environment. Furthermore, the group with high *XCR1* expression had a higher IPS than those with low *XCR1* expression. These findings suggested that the high *XCR1* group responds better to the immune checkpoint inhibitors.

Sorafenib has been an effective first-line therapy in advanced HCC patients for over ten years [31]. However, predicting therapeutic response in last-stage HCC patients receiving sorafenib is challenging [32]. Herein, we compared survival differences between the high and low *XCR1* expression groups among the patients who received sorafenib. Our results showed that high expression levels of *XCR1* were related to better OS and PFS in HCC patients treated with sorafenib. These findings suggested that patients with high *XCR1* expression are more likely to benefit from sorafenib treatment, providing guidance in drug-related decision-making for doctors.

Nevertheless, this study has several limitations. First, iCPS was scored based on one pathological slide, so it is still circumscribed due to the inevitable spatial heterogeneity of tumors. Second, Kaplan-Meier curves showed significant prognostic values of *XCR1* in the

HCC patients who received sorafenib. In contrast, a larger sample size with sorafenib or immune treatments is needed to validate its prognostic significance. In addition, the complex functions and mechanisms of *XCR1* in sorafenib treatment still need to be investigated.

5. Conclusions

In conclusion, we have shown in different datasets that *XCR1* is a valuable prognostic biomarker in the HCC population, especially in those with favorable tumor characteristics. Combining iCPS status and BCLC status has a synergistic effect on stratifying patients' OS and RFS. Further analyses showed that *XCR1* is a potential predictor for sorafenib and immune therapeutic responses.

Ethics statement

The study was conducted by the Declaration of Helsinki and the protocol was approved by the Ethics Committee of the Zhongshan Hospital, Fudan University (Approval number: B2023-147R; Approval data: 19/6/2023).

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Data availability statement

The data used for bioinformatic analysis supporting this study's findings are available in the TCGA (https://gdc.cancer.gov) and ICGC databases. All other data can be provided upon reasonable request to the corresponding authors.

Consent to participate

Informed consent was obtained from all subjects involved in this study.

Consent to publish

The authors declare that they agree to submit the article for publication.

CRediT authorship contribution statement

Wei Wu: Writing – original draft, Visualization, Validation, Methodology, Formal analysis. **Zhen Bao:** Writing – original draft, Visualization, Validation, Resources, Methodology, Formal analysis. **Kai Zhu:** Writing – original draft, Visualization, Validation, Resources, Methodology, Funding acquisition, Formal analysis. **Danjun Song:** Writing – review & editing, Visualization, Software, Methodology, Funding acquisition, Formal analysis, Data curation. **Weijian Yang:** Writing – original draft, Investigation. **Jun Luo:** Writing – review & editing, Investigation. **Jiaping Zheng:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Guoliang Shao:** Writing – review & editing, Supervision, Conceptualization. **Junfeng Huang:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Danjun Song reports financial support was provided by Zhejiang Medical and Health Science and Technology Plan Project, grant number 2023KY593, 2024KY787. Jiaping Zheng reports financial support was provided by Zhejiang Medical and Health Science and Technology Plan Project, grant number 2023KY600, 2022KY118. Danjun Song reports financial support was provided by the Cultivating funding of the Cancer Hospital of the University of Chinese Academy of Sciences, grant number PY2021025. Kai Zhu reports financial support was provided by Natural Science Funds of Shanghai, grant numbers 21ZR1413800 and 22ZR1400100.

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Appendix A. Supplementary data

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Abbreviations

XCR1	X–C motif chemokine receptor 1
HCC	Hepatocellular carcinoma
TCGA	The Cancer Genome Atlas
ICGC	International Cancer Genome Consortium
IHC	Immunohistochemistry
OS	Overall survival
RFS	Recurrence-free survival
iCPS	Immune cell proportion score
XCL1	X–C Motif Chemokine Ligand 1
RCC	Renal cell carcinoma
AJCC	American Joint Committee on Cancer
IPS	Immunophenoscore
MHC	Major histocompatibility complex
TMB	tumor mutational burden
EMT	Epithelial-Mesenchymal Transition

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