Original Article

Iranian Journal of Immunology

March 2025; 22(1):

CX3CR1 Functions as a Biomarker Associated with Pathological Tumor Staging in the Diagnosis and Prognosis of Prostate Cancer

Shiquan Xu[1*], Qing Liu[1*], Jie Wang[1], Zeyun Zhang[1], Ying Wang[1], Li Li[1], Tao Zhang[1#], Yu Fan[1]

[1]Department of Surgery, Xiang'an Hospital of Xiamen University, School of Medicine, Xiamen University, Xiamen 361102, China
#They contribute equal to this work.

ABSTRACT

Background: Previous research has identified several potential biomarkers associated with pathological tumor (pT) staging in prostate cancer (PCa) patients. Among these biomarkers, CX3CR1 is notable for its connection to the immune microenvironment.

Objective: To further investigate the significance of CX3CR1 as a key biomarker for predicting pT staging and PCa progression.

Methods: Prostate cancer tissue samples were analyzed using quantitative reverse transcription polymerase chain reaction (qRT-PCR) and immunohistochemical staining. The diagnostic performance of CX3CR1 was evaluated using receiver operating characteristic (ROC) curves, while Kaplan–Meier survival analysis was conducted to determine overall survival (OS) rates.

Results: A significant decrease in CX3CR1 expression was observed in PCa tissues compared to adjacent normal tissues, with the lowest levels detected in pT3 tumors. CX3CR1 expression showed a negative correlation with preoperative prostate-specific antigen (PSA) levels, lymph node staging (N stage), Gleason score, and overall survival (OS). Additionally, CX3CR1 levels were associated with the polarization of infiltrating CD4+ T cells in PCa patients.

Conclusion: CX3CR1, as a biomarker associated with pT staging, plays a role in predicting PCa prognosis, potentially by modulating the immune microenvironment.

Keywords: Immune cell infiltrates, Pathological tumor staging, Prognosis, Prostate cancer, T-cell polarization

*Corresponding author:
Tao Zhang,
Department of Surgery,
Xiang'an Hospital of Xiamen University, School of Medicine, Xiamen University, Xiamen 361102, China
Email:
zhangtao19880301@163.com

Cite this article as:
Xu S, Liu Q, Wang J, Zhang Z, Wang Y, Li L, Zhang T, Fan Y. CX3CR1 Functions as a Biomarker Associated with Pathological Tumor Staging in the Diagnosis and Prognosis of Prostate Cancer. *Iran J Immunol.* 2025; doi: 10.22034/iji.2025.104018.2876.

Received: 2024-09-10
Revised: 2025-01-27
Accepted: 2025-03-10

INTRODUCTION

Pathological tumor (pT) staging categorizes cancer into early-stage (≤pT2) and advanced-stage (≥pT3) classifications based on tumor size, invasion depth, and spread (1). In prostate cancer (PCa), pT staging plays a crucial role in assessing disease severity, guiding therapeutic strategies, and estimating patient outcomes (2). However, this staging system has certain limitations, as it heavily relies on imaging and biopsy findings, which may not always provide a precise reflection of tumor aggressiveness. Consequently, staging discrepancies can occur, leading to either underestimation or overestimation of disease severity. To enhance the accuracy of pT staging and improve PCa diagnosis and management, identifying reliable biomarkers is of great importance. Additionally, since pT staging is closely linked to PCa progression and prognosis, previous studies have sought to identify tumor markers associated with pT staging (3).

Through an analysis of publicly available RNA sequencing (RNA-Seq) data (accession number GSE69223), researchers identified 14 potential markers associated with pT staging in PCa. This dataset included samples from 10 patients with pT2 PCa and five with pT3 PCa (4). Among the identified genes, CENPI, SLC38A11, ANO6, and KANK2 demonstrated prognostic relevance for PCa, validating the approach used in this study. However, CX3CR1 emerged as a particularly compelling candidate due to its strong association with tumor immunity.

CX3CR1 is a chemokine receptor expressed on various immune cells (5). Its ligand, CX3CL1 (fractalkine), exists in both membrane-bound and soluble forms (5). CX3CR1 is involved in immune cell adhesion, migration, and survival (6) and participates in multiple physiological and pathological processes, such as inflammation (7) and tumor progression (8).

In PCa, CX3CR1 has been implicated in metastasis and resistance to chemotherapy. Hypoxic conditions regulate its expression via hypoxia-inducible factor-1 and nuclear factor-kappa B, highlighting the potential of CX3CR1 signaling as a therapeutic target to mitigate metastasis initiation and progression (9, 10). Given these critical roles, this study aims to evaluate the potential of CX3CR1 as a key biomarker for predicting pT staging and PCa progression.

MATERIALS AND METHODS

Clinical Participants

The study was approved by the ethics committees of both institutions (approval number: XAHLL2024027). All participants provided written informed consent. A previous study included 87 patients with PCa and 51 with benign prostatic hyperplasia (BPH) as negative controls (4). This study is an extension of the previous research and utilized the same cohort. Formalin-fixed, paraffin-embedded (FFPE) specimens were used for clinical immunohistochemistry (IHC).

IHC

IHC staining procedures were conducted following previously established protocols (11, 12). FFPE prostate cancer tissue samples were sliced into uniform 4-μm sectionss. Two sets of slides were prepared for each sample: one set underwent standard hematoxylin and eosin (H&E) staining, while the other set was used for IHC staining of selected biomarkers. IHC expression levels were assessed using integrated optical density (IOD) measurements (11, 12). Initially, positive staining areas were identified under low magnification (×100). From these, four randomly selected, non-overlapping microscopic fields (magnification ×400) were analyzed to calculate IOD values.

The primary antibodies used in this study were CX3CR1 (#702321), CD20 (#53-0202-82), CD8 (#MA5-13473), CD4 (#MA5-32166), and CD163 (#MA5-11458), all obtained from Invitrogen (Thermo Fisher Scientific, Waltham, MA, USA).

Additionally, the antibody for tumor necrosis factor-alpha (TNF-α; 60291-1-Ig) was sourced from Proteintech (Wuhan, China).

Grouping of the CX3CR1-high and CX3CR1-low expression groups

The CX3CR1 expression data of all 87 patients with PCa were sorted from low to high, and the median (i.e., the 50th percentile) was used as the cutoff point. Patients with expression levels above the median were classified into the CX3CR1-high group, while patients with expression levels below the median were classified into the CX3CR1-low group.

RNA-Seq Analysis

Following the results of IHC, three PCa FFPE tissue samples with high levels of CX3CR1 and three PCa FFPE tissue samples with low levels of CX3CR1were chosen for RNA-Seq analysis. RNA extraction was carried out using the RNeasy FFPE Kit (Qiagen, Hilden, Germany). Library preparation and RNA-Seq were conducted by BioGenius (Shanghai, China, https://www.biogenius.cn/). Gene Ontology analysis using Metascape was utilized to identify differentially expressed transcripts (13).

Quantitative Reverse-transcription Polymerase Chain Reaction (qRT-PCR)

Deparaffinization of FFPE tissue and RNA extraction were performed as previously described (14). Subsequently, qRT-PCR was conducted using the GAPDH mRNA levels as an internal control to normalize the data. The primers utilized in this study are listed below:
BCL-6:
Forward:
GGAGTCGAGACATCTTGACTGA
Reverse: ATGAGGACCGTTTTATGGGCT
IL17A:
Forward: TCCCACGAAATCCAGGATGC
Reverse:
GGATGTTCAGGTTGACCATCAC
CXCR3:
Forward: CCACCTAGCTGTAGCAGACAC
Reverse: AGGGCTCCTGCGTAGAAGTT
FOXP3:
Forward: GTGGCCCGGATGTGAGAAG
Reverse: GGAGCCCTTGTCGGATGATG
GAPDH:
Forward:
GGAGCGAGATCCCTCCAAAAT
Reverse:
GGCTGTTGTCATACTTCTCATGG

Statistical Analysis

All statistical analyses were conducted using SPSS software (IBM Corp., Armonk, NY, USA). Data were presented as mean ± standard deviation based on at least three independent experiments. Prior to analysis, variance homogeneity was evaluated for continuous variables. If variances were found to be homogeneous, comparisons between groups were made using two-tailed Student's *t*-tests. Otherwise, the Mann–Whitney *U* test was utilized. Statistical significance was established at $P < 0.05$. Categorical variables were examined using the Chi-square test to identify associations. Receiver operating characteristic (ROC) curve analysis was utilized to assess diagnostic accuracy, while overall survival (OS) was evaluated using Kaplan–Meier survival curves.

RESULTS

Levels of CX3CR1 in PCa Are Significantly Reduced and Have a Negative Correlation with pT Staging

The clinical characteristics of 87 patients with PCa and 51 BPHs were previously reported (4). CX3CR1 expression levels were evaluated using IHC in a cohort of 87 PCa patients as well as 51 BPH samples. The findings revealed a significant decrease in CX3CR1 expression in PCa tissues (Fig. 1A–B). Furthermore, CX3CR1 levels showed a negative correlation with pathological tumor (pT) staging (Fig. 1C). ROC analysis indicated that CX3CR1 expression could effectively distinguish between PCa patients

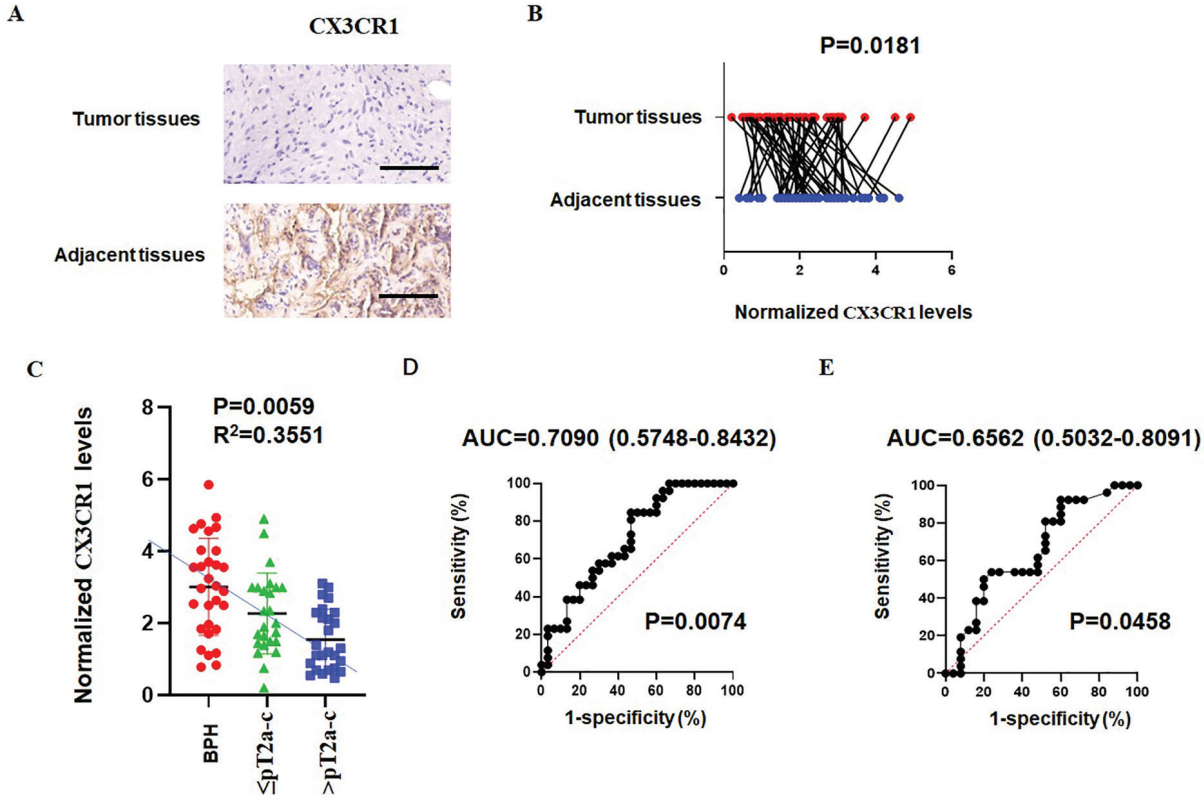

Fig. 1. Validation of CX3CR1 as a promising pT staging-associated biomarker. (A) Representative immunohistochemical staining visual field for CX3CR1 in prostate cancer (PCa) and matched adjacent tissues. Scale bar: 50 μm. (B) Comparison of CX3CR1 expression levels between PCa and matched adjacent tissues in patients with PCa. (C) Linear correlation between CX3CR1 expression levels and pathological tumor (pT) staging. (D) Receiver operating characteristic (ROC) curve of CX3CR1 expression levels to distinguish PCa tissues from adjacent tissues. (E) ROC curve of CX3CR1 expression levels to distinguish pT3 PCa tissues from pT2 PCa tissues.

and negative controls (Fig. 1D). Additionally, it was able to differentiate between patients with PCa at stages >pT2a–c and those at ≤pT2a–c, as shown by the area under the curve (AUC) and corresponding 95% confidence interval highlighting its diagnostic potential (Fig. 1E).

The Expression Levels of CX3CR1 Are Negatively Correlated with the Clinical Characteristics and Prognosis of Patients with PCa

Patients with PCa were divided into high (40 cases) and low (47 cases) CX3CR1 expression groups. Patients with the CX3CR1-low phenotype had higher preoperative prostate-specific antigen levels, more advanced N staging, and higher Gleason scores than those with the CX3CR1-high phenotype (Table 1). Kaplan–Meier analysis indicated that low CX3CR1 expression levels were associated with worse OS (Fig. 2).

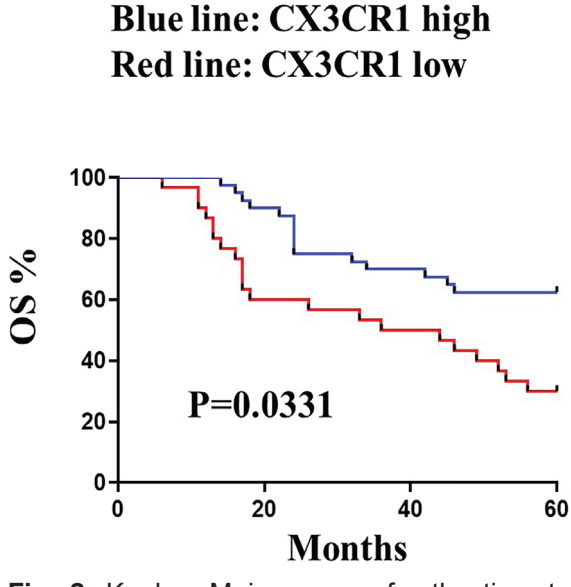

Fig. 2. Kaplan–Meier curves for the time to overall survival of patients with prostate cancer based on their CX3CR1 expression levels

Table 1. Correlation of CX3CR1 levels in PCa patients with the clinical characteristics.

	CX3CR1 phenotype		
	High (n=40)	Low (n=47)	P*
Age			
≤65 years	21	17	0.1259
>65 years	19	30	
BMI			
≤25 kg/m^2	18	22	0.866
>25 kg/m^2	22	25	
PSA			
≤10 ng/ml	16	9	0.0322
>10 ng/ml	24	38	
N stage			
N0	33	28	0.0199
N1	7	19	
Gleason score			
<7	20	10	0.00497
≥7	20	37	

*Chi-square test

CX3CR1 Expression Levels Are Associated with Primary Immune Cell Infiltration in Patients with PCa

Next, the levels of immune cell markers in PCa tissue samples were determined using IHC, and their correlation with CX3CR1 levels was plotted. A significant positive correlation was observed between CX3CR1 and CD163 (macrophage M2 marker, Fig. 3A), TNF-α (macrophage M1 marker, Fig. 3B), and CD4 levels (CD4+ T cell marker, Fig. 3C). However, no significant correlations were observed between CX3CR1 levels and CD20 (B cell marker, Fig. 3D) and CD8 levels (CD8+ T cell marker, Fig. 3E). Since CX3CR1 regulates M1/M2 macrophage polarization (5), the association between CX3CR1 levels and CD4+T cell polarization was investigated.

Correlation between CX3CR1 Levels and Infiltrated CD4+ T-cell Polarization in Patients with PCa

Three CX3CR1-high PCa tissue samples and three CX3CR1-low PCa tissue samples (from a total of 87 PCa samples) were randomly selected for subsequent RNA-Seq analysis. Several differentially expressed transcripts closely related to tumor immune infiltration were identified (Fig. 4A–B). qRT-PCR results demonstrated that BCL-6, a marker for follicular T-helper [TFH] cells, Fig. 4C), IL17A, a marker forT helper type 17 [Th17], Fig. 4D), and CXCR3 (highly expressed in T helper type 1 [Th1] cells; Fig. 4E) were enhanced. In contrast, FOXP3, a marker for regulatory T cell [Treg] cells; Fig. 4F) was decreased in the CX3CR1-high group, indicating an association between CX3CR1 and infiltrated CD4 T-cell polarization in patients with PCa.

DISCUSSION

pT staging allows for more precise personalization of treatment and provides important prognostic information, aiding in informed decision-making and improving patient outcome in the management of PCa (15, 16). While pT staging is a useful tool for evaluating PCa, it has limitations because it relies on anatomical criteria. This classification system frequently does not fully capture the biological complexity of tumors, as it does not consider molecular and genetic factors that impact tumor behavior. By primarily focusing on tumor size and anatomical spread, pT staging may overlook critical prognostic

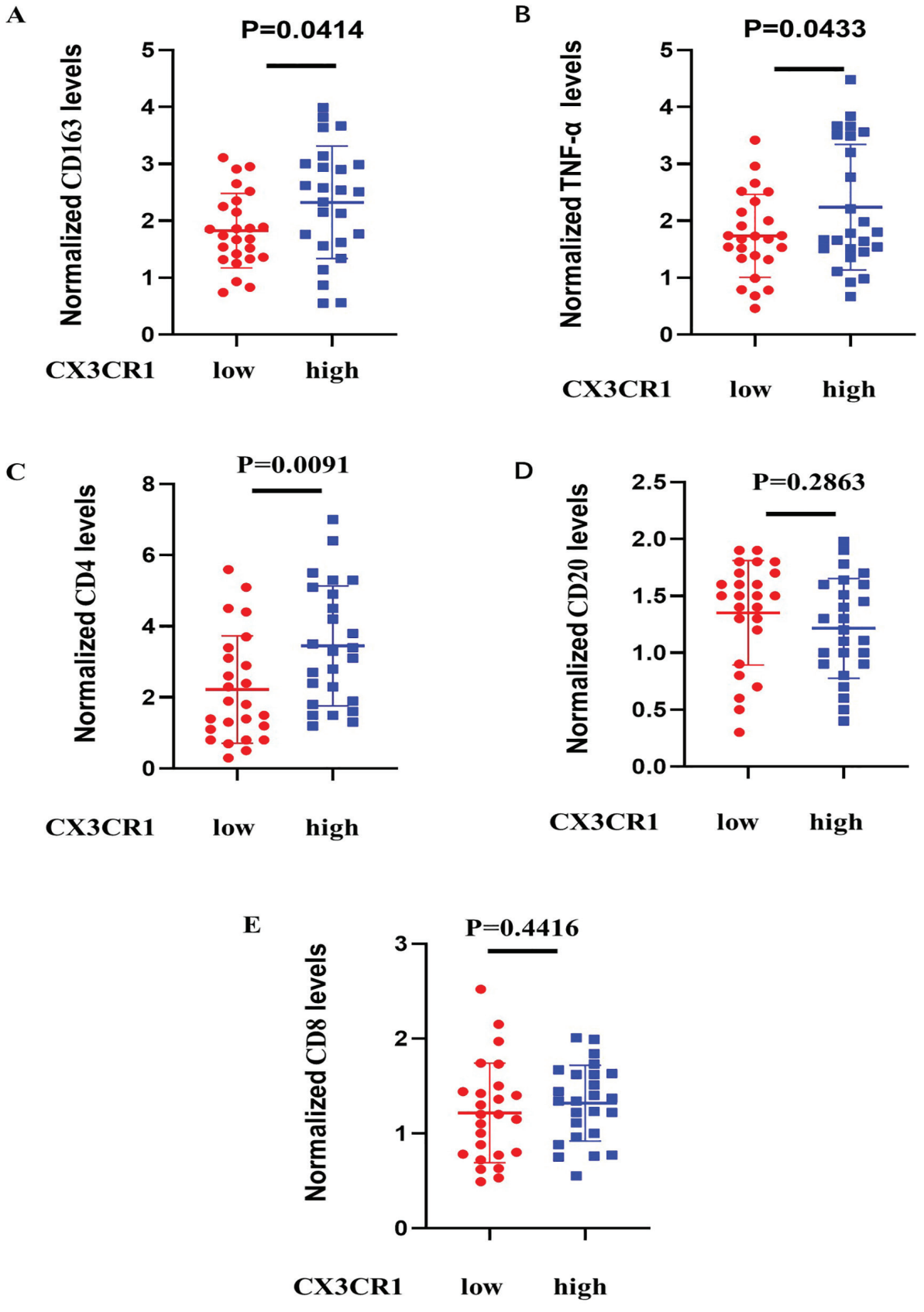

Fig. 3. Association between CX3CR1 expression levels and infiltrating immune cells. The infiltrating immune cell markers were identified through immunohistochemistry of PCa tissues. The correlations between (A) CX3CR1 and CD163; (B) CX3CR1 and tumor necrosis factor-alpha (TNF-α); (C) CX3CR1 and CD4; (D) CX3CR1 and CD20; and (E) CX3CR1 and CD8 were determined.

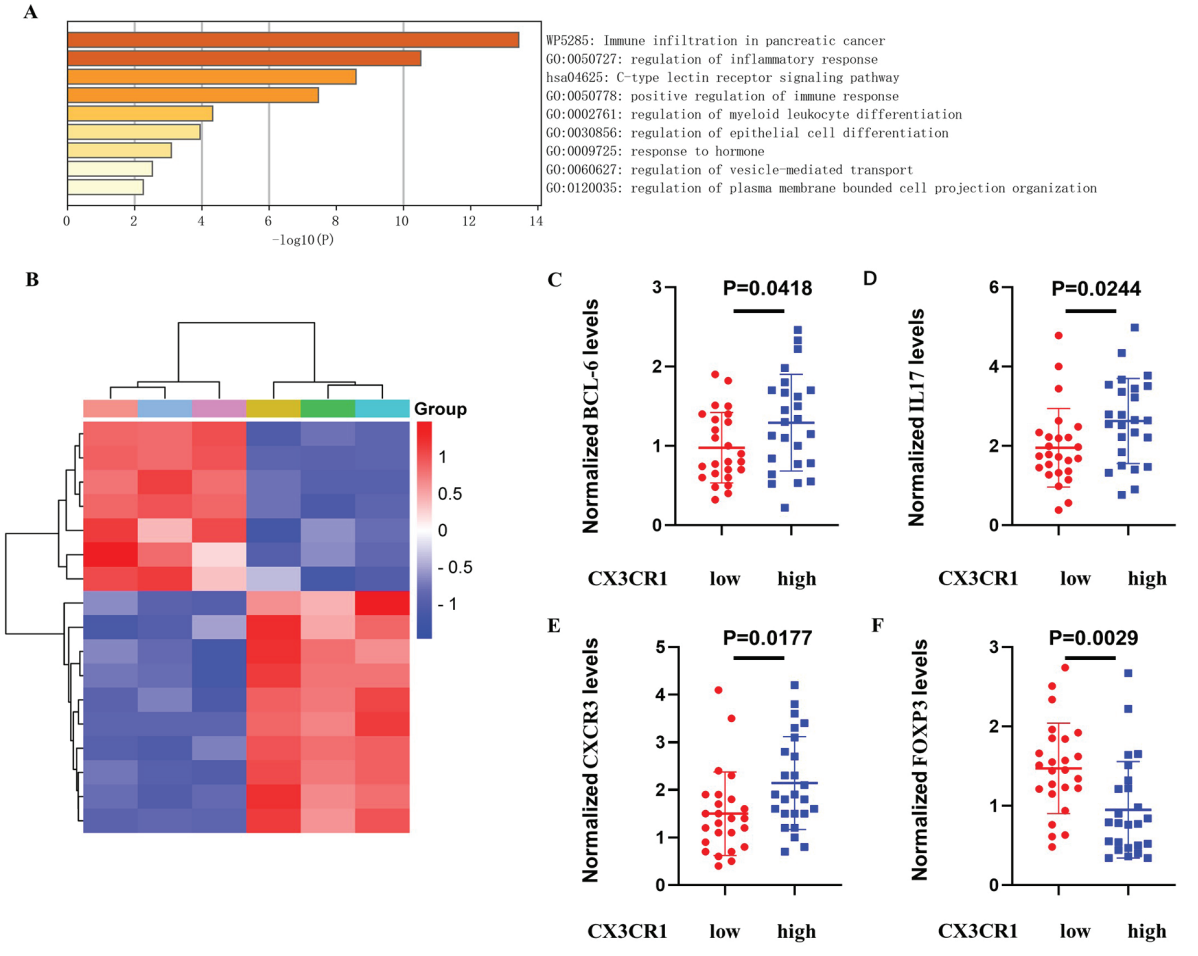

Fig. 4. Association between CX3CR1 levels and infiltrated CD4+ T-cell polarization. (A–B) Three CX3CR1-high PCa tissue samples and three CX3CR1-low PCa tissue samples were randomly selected (from the 87 PCa tissue samples), and RNA sequencing was performed. (A) The top-level Gene Ontology (GO) biological processes of the differentially expressed transcripts between the CX3CR1-high and CX3CR1-low groups. The plot was determined and drawn using Metascape Gene List Analysis. (B) A heatmap is presented, showing the differentially expressed transcripts involved in CD4+ T-cell polarization. (C–F) CD4+ T-cell polarization markers were determined using quantitative reverse transcription polymerase chain reaction of the PCa tissues. The correlation between (C) CX3CR1 and BCL-6; (D) CX3CR1 and IL17A; (E) CX3CR1 and CXCR3; and (F) CX3CR1 and FOXP3 were determined.

indicators. Additionally, it does not consider variations in tumor responses to specific treatments (17). These limitations emphasize the need for supplementary methods, such as molecular profiling, to provide a more comprehensive understanding of PCa biology. Incorporating such approaches could improve risk stratification and aid in the development of more tailored treatment strategies.

The GSE40275 dataset contains gene expression profiles from PCa tissues across different pT stages. In this study, biomarker identification was initiated by comparing differentially expressed genes between pT2 and normal tissues, as well as between pT3 and pT2 tissues, with a focus on overlapping genes to enhance the reliability of pT stage-associated marker selection. Bioinformatics analysis, combined with experimental validation, revealed a negative correlation between CX3CR1 expression and pT stage. Reduced CX3CR1 levels were also linked to more advanced clinical characteristics and poorer prognosis in PCa patients, underscoring its potential as a prognostic biomarker.

CX3CR1 has been implicated in tumor progression across multiple cancer types. For example, Yue et al. demonstrated that CX3CR1 facilitates immune cell recruitment and macrophage polarization, establishing its prognostic relevance in colorectal cancer (18). Moreover, Hu et al. identified an enrichment of CD16+CX3CR1+ monocytes in cases showing a "major pathological response" among non-small cell lung cancer patients undergoing neoadjuvant programmed death-1 blockade combined with chemotherapy (19). Similarly, Shao et al. reported that CX3CR1 plays a role in reshaping the immune microenvironment in epithelial ovarian cancer, suggesting its potential as both an immunotherapy target and a prognostic marker (20). Collectively, these findings support CX3CR1's role as a diagnostic biomarker through its modulation of immune cell function.

Immune cells, such as Th1, Th17, TFH, and Treg cells, have distinct roles in the progression of PCa and its pT staging. For example, Th1 cells trigger anti-tumor immune responses by releasing cytokines like interferon-γ and are typically linked to tumor suppression, showing increased activity in early-stage PCa (21). However, the functions and expression patterns of Th2, Th17, TFH, and Treg cells throughout tumor progression still require further investigation. The balance among these immune cells can substantially impact the clinical outcomes of PCa. This study suggests that CX3CR1 expression is closely linked to CD4+T cell polarization. Specifically, CX3CR1 expression is positively correlated with Th1, Th17, and TFH cells while negatively correlated with Treg cells. This indicates its potential role in PCa progression by regulating CD4+ T cell polarization.

A close relationship between CX3CR1 and macrophage polarization was observed. Macrophages with varying polarization patterns secrete different inflammatory factors (22), which interact with T or tumor cells (23). These findings suggest that CX3CR1 plays a crucial role in facilitating crosstalk between macrophages and T cells, contributing to the tumor microenvironment.

ACKNOWLEDGEMENT

This work was supported by the Starting Package of Xiang'an Hospital of Xiamen University (NO. PM0428 and NO. XM01460001); The project was also supported by the Natural Science Foundation of Fujian Province of China (No.2023J01014).

ETHICAL STATEMENT

All experiments were conducted in accordance with the principles of the Helsinki Declaration. This study received approval from Xiang'an Hospital of Xiamen University Hospital and Qingdao Hospital, University of Health and Rehabilitation Sciences.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Marshall CH, Fu W, Wang H, Baras AS, Lotan TL, Antonarakis ES. Prevalence of DNA repair gene mutations in localized prostate cancer according to clinical and pathologic features: association of Gleason score and tumor stage. Prostate Cancer Prostatic Dis. 2019;22(1):59-65.
2. Haug ES, Myklebust TÅ, Juliebø-Jones P, Reisæter LAR, Aas K, Berg AS, et al. Impact of prebiopsy MRI on prostate cancer staging: Results from the Norwegian Prostate Cancer Registry. BJUI Compass. 2023;4:331-338.
3. Krstanoski Z, Vokac NK, Zagorac A, Pospihalj B, Munda M, Dzeroski S, Golouh R. TMPRSS2:ERG gene aberrations may provide insight into pT stage in prostate cancer. BMC Urol. 2016;16:35.
4. Xu S, Shi H, Liu Y, Lin J, Wu X, Lu R, et al. Identification of biomarkers associated with pathological tumor staging and their utility in the diagnosis and prognosis of prostate cancer. Lab Med. 2024:lmae059.
5. Ni Y, Zhuge F, Ni L, Nagata N, Yamashita T,

Mukaida N, Kaneko S, Ota T, Nagashimada M. CX3CL1/CX3CR1 interaction protects against lipotoxicity-induced nonalcoholic steatohepatitis by regulating macrophage migration and M1/M2 status. Metabolism. 2022;136:155272.
6. Lee M, Lee Y, Song J, Lee J, Chang SY. Tissue-specific Role of CX_3CR1 Expressing Immune Cells and Their Relationships with Human Disease. Immune Netw. 2018;18:e5.
7. Szukiewicz D. CX3CL1 (Fractalkine)-CX3CR1 Axis in Inflammation-Induced Angiogenesis and Tumorigenesis. Int J Mol Sci. 2024;25:4679.
8. Lu X. Structure and Function of Ligand CX3CL1 and its Receptor CX3CR1 in Cancer. Curr Med Chem. 2022;29:6228-6246.
9. DiNatale A, Kaur R, Qian C, Zhang J, Marchioli M, Ipe D, Castelli M, McNair CM, Kumar G, Meucci O, Fatatis A. Subsets of cancer cells expressing CX3CR1 are endowed with metastasis-initiating properties and resistance to chemotherapy. Oncogene. 2022;41:1337-1351.
10. Xiao LJ, Chen YY, Lin P, Zou HF, Lin F, Zhao LN, Li D, Guo L, Tang JB, Zheng XL, Yu XG. Hypoxia increases CX3CR1 expression via HIF-1 and NF-κB in androgen-independent prostate cancer cells. Int J Oncol. 2012;41:1827-1836.
11. Singh G, Sankanagoudar S, Dogra P, Chandra NC. Interlink between cholesterol & cell cycle in prostate carcinoma. Indian J Med Res. 2017;146(Suppl):S38-S44.
12. Ni Q, Sun J, Ma C, Li Y, Ju J, Sun M. The Neuropilins and Their Ligands in Hematogenous Metastasis of Salivary Adenoid Cystic Carcinoma-An Immunohistochemical Study. J Oral Maxillofac Surg. 2018;76:569-579.
13. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, Benner C, Chanda SK. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun. 2019;10:1523.
14. Park S, Wang HY, Kim S, Ahn S, Lee D, Cho Y, et al. Quantitative RT-PCR assay of HER2 mRNA expression in formalin-fixed and paraffin-embedded breast cancer tissues. Int J Clin Exp Pathol. 2014;7:6752-6759.
15. Haug ES, Myklebust TÅ, Juliebø-Jones P, Reisæter LAR, Aas K, Berg AS, et al. Impact of prebiopsy MRI on prostate cancer staging: Results from the Norwegian Prostate Cancer Registry. BJUI Compass. 2023;4:331-338.
16. Vetrone L, Mei R, Bianchi L, Giunchi F, Farolfi A, Castellucci P, et al. Histology and PSMA Expression on Immunohistochemistry in High-Risk Prostate Cancer Patients: Comparison with 68Ga-PSMA PET/CT Features in Primary Staging. Cancers (Basel). 2023;15:1716.
17. Cosma G, Acampora G, Brown D, Rees RC, Khan M, Pockley AG. Prediction of Pathological Stage in Patients with Prostate Cancer: A Neuro-Fuzzy Model. PLoS One. 2016 Jun 3;11(6):e0155856.
18. Yue Y, Zhang Q, Sun Z. CX3CR1 Acts as a Protective Biomarker in the Tumor Microenvironment of Colorectal Cancer. Front Immunol. 2022;12:758040.
19. Hu J, Zhang L, Xia H, Yan Y, Zhu X, Sun F, Sun L, Li S, Li D, Wang J, Han Y, Zhang J, Bian D, Yu H, Chen Y, Fan P, Ma Q, Jiang G, Wang C, Zhang P. Tumor microenvironment remodeling after neoadjuvant immunotherapy in non-small cell lung cancer revealed by single-cell RNA sequencing. Genome Med. 2023;15(1):14.
20. Shao D, Zhou H, Yu H, Zhu X. CX3CR1 is a potential biomarker of immune microenvironment and prognosis in epithelial ovarian cancer. Medicine (Baltimore). 2024;103:e36891.
21. Silva RCMC, Lopes MF, Travassos LH. Distinct T helper cell-mediated antitumor immunity: T helper 2 cells in focus. Cancer Pathog Ther. 2022;1:76-86.
22. Wang D, Lin Y, Xu F, Zhang H, Zhu X, Liu Z, et al. SIRPα maintains macrophage homeostasis by interacting with PTK2B kinase in Mycobacterium tuberculosis infection and through autophagy and necroptosis. EBioMedicine. 2022;85:104278.
23. Wu X, Lu W, Xu C, Jiang C, Zhuo Z, Wang R, et al. Macrophages Phenotype Regulated by IL-6 Are Associated with the Prognosis of Platinum-Resistant Serous Ovarian Cancer: Integrated Analysis of Clinical Trial and Omics. J Immunol Res. 2023;2023:6455704.