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Role of USP10/METTL3/CXCR4 Axis in Immunotherapy of Castration-Resistant Prostate Cancer

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ABSTRACT

The aim of this study was to investigate the role of the ubiquitin specific peptidase 10 (USP10)/methyltransferase like 3 (METTL3)/C-X-C chemokine receptor type 4 (CXCR4) axis in immunotherapy of castration-resistant prostate cancer (CRPC).

Knockdown experiments were conducted in CRPC cell lines to assess the effect of targeting CXCR4 on cell proliferation invasion and migration. Coculture experiments of *CXCR4* knockdown CRPC cells with THP1-M0 were performed to evaluate their impact on macrophage polarization and migration ability. With $CD8^+$ T cells was conducted to assess their effects on $CD8^+$ T cell proliferation and apoptosis. *CXCR4*-overexpressing CRPC cells were treated with the JAK-2 specific inhibitor AG490 to assess the effect of *CXCR4* through the JAK2/STAT3 pathway on CRPC. The mechanisms by which USP10 regulates *CXCR4* expression through targeting METTL3 were explored through dataset analysis, bioinformatics prediction, and Western blot.

In CRPC tissues and cells, there was an observed increase in *CXCR4* expression. Suppressing *CXCR4* through knockdown methods resulted in the inhibition of CRPC cell growth, movement, and infiltration. Additionally, it led to a reduction in M2 polarization and the recruitment of Tohoku Hospital Pediatrics-1 (THP1) M0 macrophages, along with a mitigation of $CD8^+$ T cell exhaustion. Dataset analysis, bioinformatics prediction, and Western blot validation indicated that CXCR4 activates the JAK2/STAT3 pathway to promote the expression of CCL2 and PD-L1, while USP10 promotes *CXCR4* expression through METTL3.

Our study underscores the significance of the USP10/METTL3/CXCR4 axis in immunotherapy for CRPC and CXCR4 as a potential target for therapeutic intervention in CRPC treatment.

Keywords: Castration-resistant prostate neoplasms; CXCR4 receptor; METTL3 protein; USP10 protein

INTRODUCTION

Prostate cancer (PCa) is the second most common malignant disease in the male urinary system, with millions of new cases diagnosed worldwide every year.[1,2] The incidence of PCa in China is increasing year by year.[3] The primary treatment options for locally advanced or metastatic PCa include surgery or chemical androgen deprivation therapy (ADT), which involves strategies for androgen depletion and anti-androgen therapy.[3] However,

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the efficacy of ADT is limited as PCa cells can adapt to low androgen environments. This adaptation is primarily achieved through the induction of androgen receptor (AR) expression and signaling mechanisms. This adaptive response presents a substantial hurdle in treating PCa. Despite an initial positive reaction to ADT, patients frequently develop resistance to treatment and advance to a stage known as castration-resistant prostate cancer (CRPC).[4,5] CRPC represents the final stage of PCa and remains a major clinical challenge.[6] Identifying appropriate therapeutic strategies is crucial for improving treatment outcomes in CRPC patients.

Tumor immunotherapy has been a focal point in basic cancer research, leading to the successful development of various targeted therapies that enhance the cytotoxic effects of immune cells against tumors, yielding significant clinical outcomes.[7] However, due to the unique immunosuppressive tumor microenvironment (TME) of CRPC, the effectiveness of standalone immunotherapies is often suboptimal. Thus, identifying suitable immunotherapy regimens is crucial for improving treatment outcomes in CRPC patients. Previous studies have shown that tumor-associated macrophages (TAMs), adipocytes, and hypoxia within the TME are key factors contributing to immunosuppression and can significantly upregulate programmed cell death-Ligand 1 (PD-L1) expression in CRPC cells.[8-10] Simultaneously targeting the Janus kinase–signal transducer and activator of transcription (JAK/STAT3) pathway along with PD-L1 has demonstrated greater efficacy in boosting the cytotoxic capabilities of natural killer cells against CRPC.[11] Inhibition of ataxia telangiectasia mutated (ATM) reduces epithelial-mesenchymal transition (EMT), invasion, and metastasis in CRPC, decreases macrophage recruitment, and increases CRPC sensitivity to natural killer cell inhibitors by suppressing the CXCL12 and PI3K/AKT-PD-L1 pathways.[10] However, the expression and functional mechanisms of C-X-C chemokine receptor type 4 (CXCR4), the receptor for CXCL12, in CRPC remain unclear. Studies have indicated that high intratumoral CXCR4 mRNA expression is associated with T-cell and macrophage-enriched pancreatic cancer phenotypes and high expression of inhibitory immune checkpoints.[12] Moreover, concurrent inhibition of CXCR4 and PD-1 has been demonstrated to augment the cytotoxicity mediated by $CD8^+$ T-cells against human pancreatic cancer cells.[13] Research has shown that C-C motif chemokine ligand 2 (CCL2) and PD-L1, which act as promoters of macrophage M2 polarization and $CD8^+$ T-cell exhaustion, respectively, are regulated by the JAK2/STAT3 pathway in tumor cells.[14,15] Our research has observed that CXCR4 stimulates the upregulation of CCL2 and PD-L1 through the activation of the JAK2/STAT3 pathway, thereby contributing to macrophage M2 polarization and $CD8^+$ T-cell exhaustion. Nonetheless, the regulatory mechanisms of *CXCR4* expression during CRPC progression remain to be further elucidated.

M6A plays a crucial role in tumor occurrence and development.[16] methyltransferase like 3 (METTL3), identified as a crucial component within the methyltransferase complex, has been documented to facilitate immune evasion in diverse cancer cell types.[17] Studies have found that high levels of m6A-modified RNA exist in CRPC, primarily due to the upregulation of METTL3. METTL3 increases CRPC cell proliferation, migration, and resistance to ADT drugs such as enzalutamide by activating the extracellular-signal-regulated kinase (ERK) signaling pathway.[18] However, whether METTL3 can participate in CXCR4-mediated macrophage M2 polarization and $CD8^+$ T-cell exhaustion by regulating *CXCR4* mRNA m6A modification remains to be further explored.

Deubiquitinating enzymes (DUBs) are key regulators in the ubiquitin signaling pathway, involved in various physiological activities such as cell proliferation, cell cycle, signal transduction, inflammation, metabolism, and immunity.[19] USP10, belonging to the ubiquitin-specific protease family, exhibits the ability to deubiquitinate protein substrates by cleaving ubiquitin chains. Over the past years, mounting evidence has underscored the significant involvement of USP10 in both tumor progression and immune escape mechanisms.[20,21] In breast cancer, USP10 acts as a deubiquitinase that binds to IGF2BP1, stabilizing its protein expression through deubiquitination, which in turn promotes m6A modification on CPT1A mRNA and enhances its stability, leading to breast cancer metastasis.[22] High expression of USP10 is associated with poor prognosis in PCa patients, and its oncogenic role involves the p53-G3BP2 comple x and AR signaling.[23] However, it remains to be investigated whether USP10 can regulate *CXCR4* expression in CRPC cells through METTL3, activate the JAK2/STAT3 pathway, promote CCL2 expression to induce macrophage M2 polarization,

increase PD-L1 expression to facilitate T cell exhaustion, and ultimately play a role in the malignant progression of CRPC.

Based on this, our study focused on CRPC cell lines and CRPC tissues, particularly investigating the expression and regulatory mechanisms of CXCR4 in CRPC. Overall, our research emphasizes the significance of the USP10/METTL3/CXCR4 axis in immunotherapeutic strategies for CRPC, underscoring CXCR4 as a promising target for therapeutic intervention in CRPC.

MATERIALS AND METHODS

Data Source and Selection

We download the dataset GSE32269 on human primary PCa and CRPC from the GEO and perform bioinformatics analysis. Using the GEO2R tool to analyze differentially expressed genes in CRPC.

Cell Culture

Human normal prostate epithelial cells (RWPE) and CRPC cell lines (LNCaP, C4-2, CWR22Rv1) were sourced from American Type Culture Collection (ATCC) (Manassas, VA, USA) and maintained in RPMI1640 medium (Corning, Corning, NY, USA) supplemented with 10% FBS and 1% penicillin-streptomycin solution. Cells were cultured under standard conditions at 37℃ in a 5% CO_2 incubator.

Cell Transfection

sh-NC, sh-CXCR4, sh-USP10, and OE-METTL3 plasmid lentiviral vectors were provided by Sangon Biotech (Shanghai, China), and transfect the virus according to the instructions of Lipofectamine 3000 manufacturer (Invitrogen, Carlsbad, CA). The culture medium was replaced about 6 hours later. Forty-eight hours after transfection, the cells were used for further experiments.

EdU Assay

Inoculate logarithmic growth stage cells onto a 24-well plate, culture overnight, and transfected according to the established method. Then, treat the cells with 20 nM of DHT for 24 hours. Cells were labeled with 10 μmol/L EdU in the EdU staining kit (Thermo, Waltham, MA, USA) and incubated on the culture plate in an incubator for 2 hours. Wash cells with phosphate buffer, in each well, introduce 2 mL of 4% paraformaldehyde solution, and allow fixation to occur at room temperature for 0.5 hours. Subsequently, neutralize any remaining aldehyde groups by treating them with glycine. Subsequently, decolorize with phosphate buffer on a decolorization shaker for 6 minutes, add TritonX-100 to break the membrane for 10 minutes, and add Apollo Incubate the solution in the dark for 20 minutes, wash the cells again with phosphate buffer containing TritonX-100, and add DAPI to stain the nuclei for 15 minutes. Finally, place the 24-well plates under a microscope for observation and take photos.

Transwell Migration Assay

Inoculate logarithmic growth stage cells onto a 6-well plate with a final concentration of 1×10^5 cells/mL, culture overnight, and transfected according to the established method. Add 200 μL to the upper chamber of Transwell chamber cell suspension (1×10^5 cells/mL), and the lower chamber of Transwell chamber was supplemented with 600 μL 1640 complete medium containing 10% Fetal Bovine Serum (FBS) and double-antibody. Place the chamber in a 37℃, 5% CO_2 incubator and continue to cultivate for 24 hours. Remove the Transwell chamber and add 4% paraformaldehyde to fix for 20 minutes; Add 350 μL crystal violet dye for 10 minutes and wash with PBS three times. Employ a cotton swab to remove the cells residing on the inner surface of the Transwell chamber that haven't traversed the membrane. Subsequently, capture images using an inverted microscope.

Transwell Invasion Assay

Mix Matrigel matrix with serum-free medium in a proportion of 1:4 and apply 50 μL of the resulting diluted Matrigel onto the upper chamber of the Transwell insert. Incubate at 37℃ with 5% CO_2 for 5 hours. Transfected cells are switched to a serum-free medium 12 hours before the experiment. Place 100 μL of cell suspension, with a concentration of 3×10^4 cells/mL, into the upper chamber of the Transwell, and introduce 500 μL of medium containing 10% FBS into the lower chamber. Continue to incubate for 48 hours at 37℃ with 5% CO_2. Remove the medium from the chambers, fix the cells with 4% paraformaldehyde for 30 minutes, and use a cotton swab to clear the Matrigel and remaining cells from the upper chamber. Apply 1% crystal violet staining solution for 30 minutes, then examine the samples using an inverted microscope.

Flow Cytometry for Macrophage Polarization

Transfected cells from each group were stained with 5 μL of fluorescein isothiocyanate (FITC)-conjugated CD11b antibody and 5 μL of PC7-conjugated CD206 antibody. Flow cytometry (Beckman, Franklin Lakes, NJ, USA) was used to detect the CD206 positive rate.

Flow Cytometry for $CD8^+$ T Cell Apoptosis

After coculturing CRPC cells and $CD8^+$ T cells for 72 hours, follow the instructions of the Annexin V-FITC Apoptosis Detection Kit (Beyotime, Shanghai, China) to detect apoptosis in each group of cells using a flow cytometer (Beckman, Franklin Lakes, NJ, USA).

Western Blotting

The cells were seeded onto a 6-well plate, allowed to culture overnight, and subsequently transfected using the established protocol. After 48 hours, the old medium was discarded and pancreatic enzymes were added to digest and collect the cells. Weigh about 100 mg of benign prostatic hyperplasia (BPH), PCa, and CRPC tissue, add liquid nitrogen and protease inhibitor, and grind into powder. The cells and the ground tissues were added to 1 mL of RIPA lysate containing PMSF (Beyotime, Shanghai, China) and cleaved on ice for 30 minutes to extract the protein. sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis, membrane transfer, and subsequent protein isolation procedures were carried out. Primary antibodies targeting CXCR4, CCL2, PD-L1, JAK2, p-JAK2, STAT3, p-STAT3, USP10, and METTL3 (obtained from Cambridge, UK) were allowed to incubate overnight at 4°C. This was followed by incubation with secondary antibodies at room temperature for 1.5 hours. Protein visualization was performed with ChemiDoc-XRs+ (Bio-Rad) using beta-actin as the internal reference protein.

Real-time Quantitative Polymerase Chain Reaction (RT-qPCR)

The cells were inoculated on 6-well plates, cultured overnight, transfected as described before, and after 48 hours, the old medium was discarded and pancreatic enzymes were added to digest and collect the cells. Weigh about 100 mg BPH, PCa, and CRPC tissue, add a small amount of liquid nitrogen grinding tissue, grinding into powder. TRIzol reagent was added to both the cells and the ground tissues to extract total RNA, and then cDNA was synthesized by reverse transcription using a high-efficiency one-strand cDNA synthesis kit (TaKaRa, Tokyo, Japan). After the concentration was measured, a one-step reverse transcription real-time fluorescent quantitative PCR kit was used for reverse transcription and PCR amplification. glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal reference.

Statistical Analysis

The results were expressed as mean ± SEM and subjected to statistical analysis using GraphPad 9.0 (La Jolla, CA, USA). Variations were evaluated through two-way analysis of variance (ANOVA), with significance set at $p<0.05$. The experiment was repeated 3 times to ensure reliability.

RESULTS

Upregulation of *CXCR4* in CRPC Tissues and Cells

To investigate the expression of *CXCR4* in CRPC tissues and cells, we downloaded the dataset GSE32269 on human primary PCa and CRPC from the GEO and performed bioinformatics analysis on it, then used the GEO2R tool to analyze differentially expressed genes in CRPC. Results indicated that *CXCR4* expression is upregulated in CRPC tissues (Figure 1A). RT-qPCR was used to measure the *CXCR4* gene expression in 20 CRPC tissue samples, 20 PCa tissue samples, and 20 benign prostatic hyperplasia (BPH) specimens. The findings revealed that *CXCR4* mRNA was significantly higher in CRPC tissues compared to PCa and BPH specimens (Figure 1B). Western blot analysis provided additional confirmation of heightened CXCR4 protein levels in CRPC tissues in contrast to specimens from PCa and BPH (Figure 1C), and *CXCR4* was also highly expressed in CRPC cells (Figure 1D).

Knockdown of *CXCR4* Inhibits CRPC Cell Proliferation, Migration, and Invasion

To ascertain the potential inhibitory effects of *CXCR4* knock-down on the proliferation, migration, and invasion of CRPC cells, sh-CXCR4 was introduced into CRPC cells via transfection. Western blotting (WB) analysis revealed a significant reduction in CXCR4 protein expression following sh-CXCR4 transfection in CRPC cells (Figure 2A). EdU results showed that knocking down *CXCR4* expression significantly inhibited CRPC cell proliferation (Figure 2B). The

findings from the Transwell migration and invasion assays demonstrated a notable decrease in the migratory and invasive capabilities of CRPC cells upon *CXCR4* downregulation (Figures 2C and 2D).

Knockdown of *CXCR4* in CRPC Cells Inhibits M2 Polarization and Recruitment of THP1-M0 Macrophages

In order to verify whether CRPC cells with *CXCR4* knocked down inhibited M2 polarization and recruitment of THP1-M0 macrophages, we transfected sh-CXCR4 into CRPC cells, and WB results showed that transfection of sh-CXCR4 significantly reduced CCL2 expression in CRPC cells (Figure 3A). RT-qPCR findings indicated that CRPC cells that knocked down *CXCR4* significantly inhibited the mRNA expression of M2 markers [CD163, transforming growth factor beta 1 (TGF-β1), interleukin 10 (IL-10), and arginase 1 (ARG1)] in THP1-M0 macrophages (Figure 3B–C). FCM experiment (FCM) experiment results showed that CRPC cells with *CXCR4* knockdown significantly inhibited the expression of M2-type marker CD206 in THP1-M0 macrophages (Figure 3D–F). Transwell experiment results showed that CRPC cells that knocked down CXCR4 significantly inhibited the migration and recruitment of THP1-M0 macrophages (Figure 3G–I).

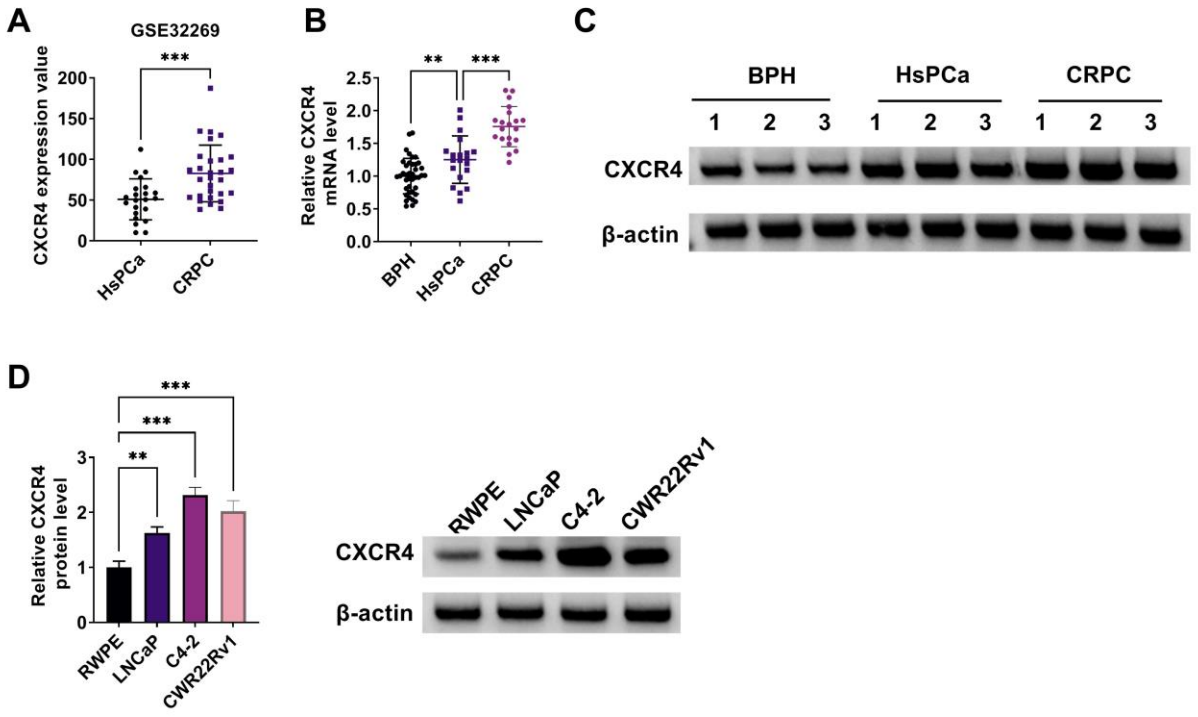

Figure 1. Heightened C-X-C chemokine receptor type 4 (CXCR4) levels in castration-resistant prostate cancer (CRPC) tissues and cells. (A) Analysis of *CXCR4* expression in CRPC tissues and cells using the Gene Expression Omnibus (GSE) 32269 dataset; (B) Reverse transcription quantitative polymerase chain reaction (RT-qPCR) detection of *CXCR4* gene expression levels in CRPC tissues, prostate cancer (PCa) tissues, and benign prostatic hyperplasia (BPH) specimens; (C) Western blot analysis of CXCR4 protein expression levels in CRPC tissues, PCa tissues, and BPH specimens; (D) Western blot analysis of CXCR4 protein expression levels in CRPC cells.

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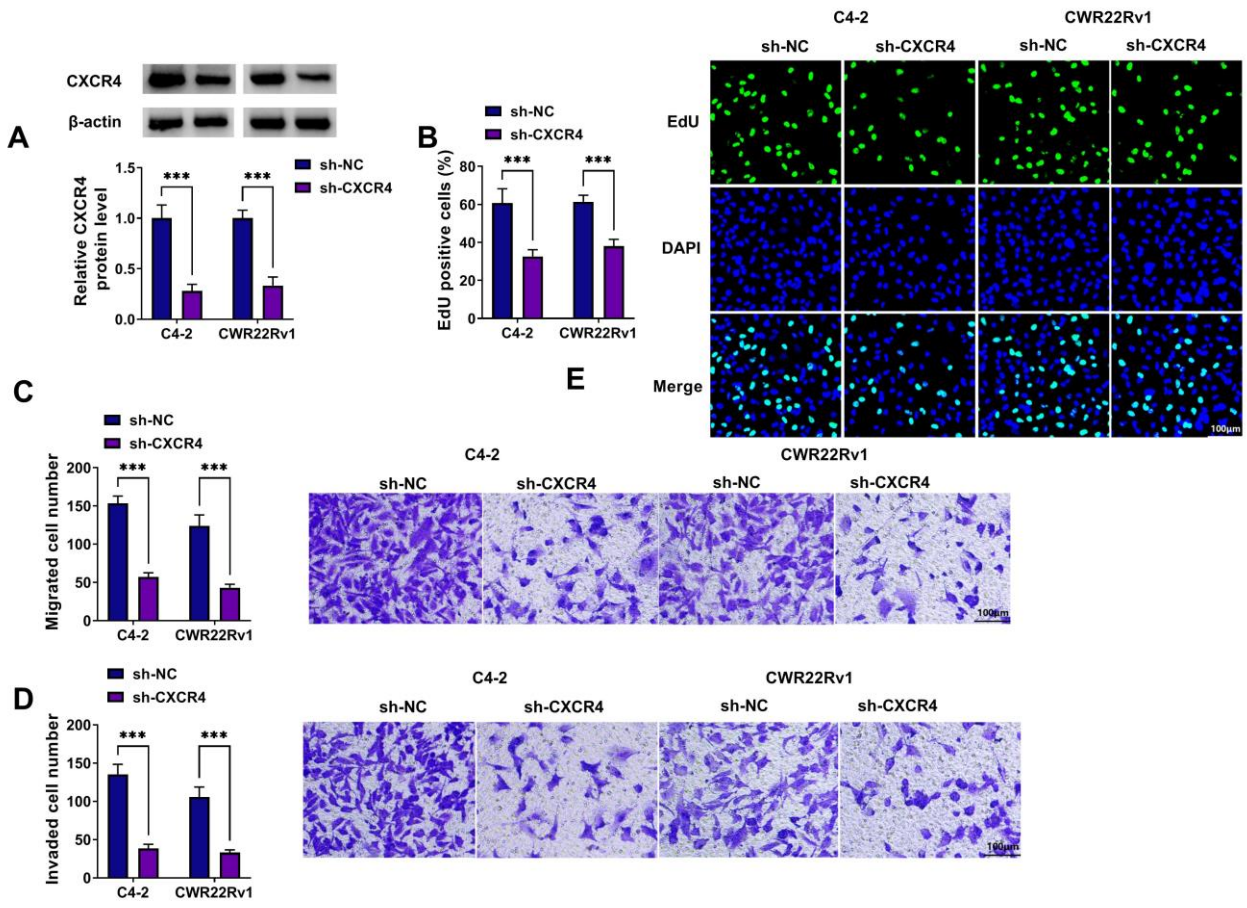

Figure 2. Knockdown of C-X-C chemokine receptor type 4 (CXCR4) inhibits castration-resistant prostate cancer (CRPC) cell proliferation, migration, and invasion. (A) Western blot analysis was conducted to assess changes in CXCR4 protein expression levels in CRPC cells following transfection with short hairpin RNA targeting CXCR4 (sh-CXCR4); (B) 5-ethynyl-2'-deoxyuridine (EdU) assay to assess the proliferation levels of CRPC cells after transfection with sh-CXCR4; (C–D) Transwell assays were performed to assess the migratory and invasive capacities of CRPC cells after sh-CXCR4 transfection.

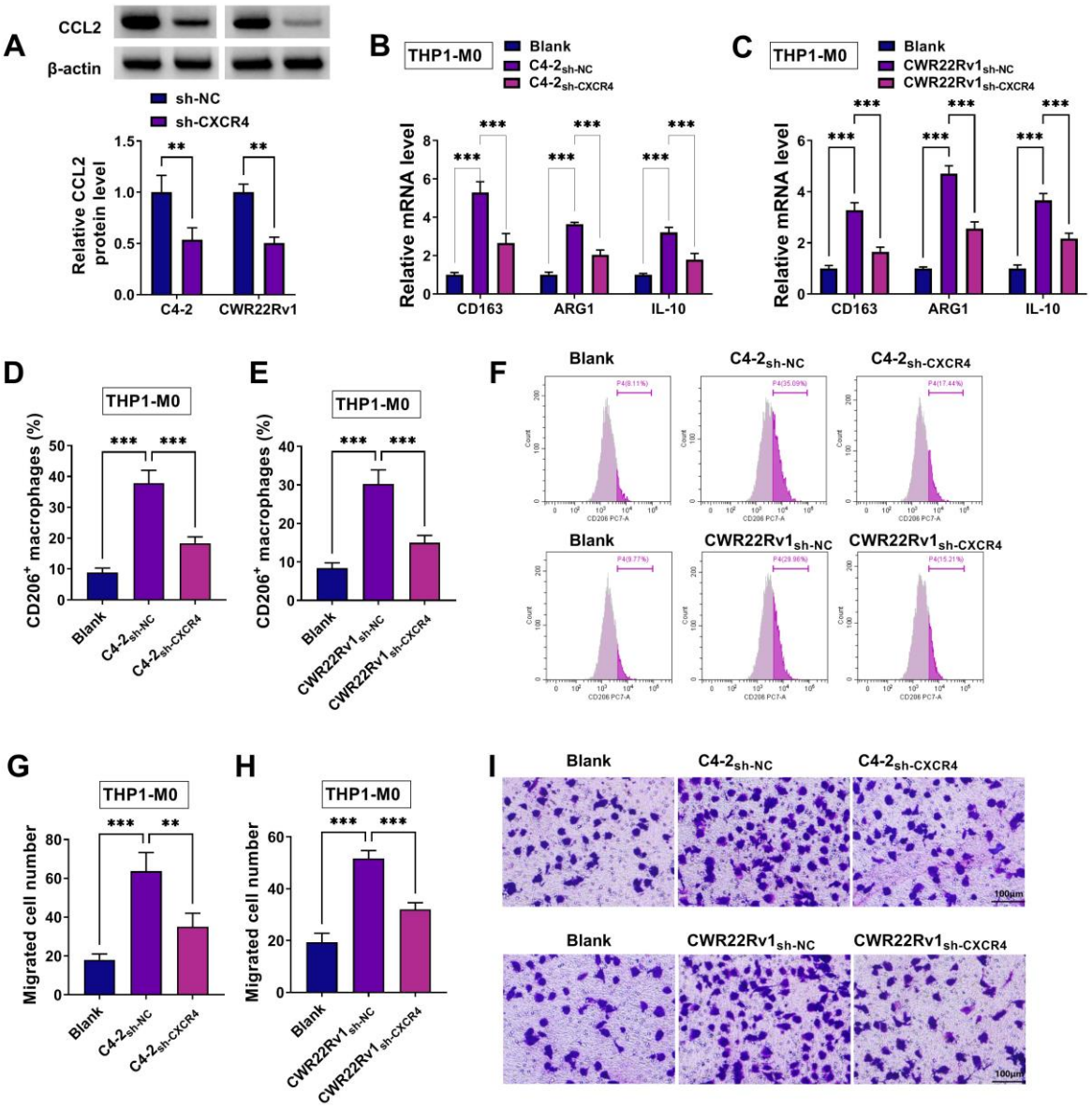

Figure 3. Castration-resistant prostate cancer (CRPC) cells that knock down C-X-C chemokine receptor type 4 (CXCR4) inhibit M2 polarization and recruitment of THP1-M0 macrophages. (A) The expression level of C-C motif chemokine ligand 2 (CCL2) protein in short hairpin RNA targeting CXCR4 (sh-CXCR4) transfected CRPC cells was detected by western blot (WB); (B–C) The levels of messenger RNA (mRNA) expression for M2 macrophage markers [cluster of differentiation 163 (CD163), transforming growth factor beta 1 (TGF-β1), interleukin 10 (IL-10), and arginase 1 (ARG1)] in CRPC cells transfected with sh-CXCR4 were detected by reverse transcription quantitative polymerase chain reaction (RT-qPCR) assay. (D–F) Flow cytometry (FCM) assay was used to detect the expression level of M2 macrophage marker cluster of differentiation 206 (CD206) in CRPC cells transfected with sh-CXCR4. The invasion and migration levels of sh-CXCR4 transfected CRPC cells were detected by (G–I) transwell assay.

Knockdown of CXCR4 in CRPC Cells Inhibits CD8$^+$ T Cell Exhaustion

To verify whether the knockdown of *CXCR4* in CRPC cells inhibits CD8$^+$ T cell exhaustion, CRPC cells were transfected with sh-CXCR4. Western blot analysis showed that transfection with sh-CXCR4 significantly reduced PD-L1 protein expression in CRPC cells (Figure 4A). CFSE staining results indicated that coculturing CD8$^+$ T cells with sh-NC transfected CRPC cells reduced the proliferation capacity of CD8$^+$ T cells, whereas knockdown of *CXCR4* in CRPC cells significantly promoted CD8$^+$ T cell proliferation (Figures 4B and 4C). Flow cytometry analysis demonstrated that knockdown of CXCR4 in CRPC cells markedly enhanced CD8$^+$ T cell apoptosis (Figures 4D–F).

Activation of the JAK2/STAT3 pathway by CXCR4 enhances the expression of CCL2 and PD-L1

Next, we transfected CRPC cells with OE-CXCR4 and simultaneously treated them using the JAK2-specific inhibitor AG490. WB results showed that OE-CXCR4 could significantly increase the protein levels of p-JAK2, p-STAT3, PD-L1, and CCL2 in CRPC cells. This promoting effect was partially reversed upon treatment using the JAK2-specific inhibitor AG490 (Figures 5A and 5B).

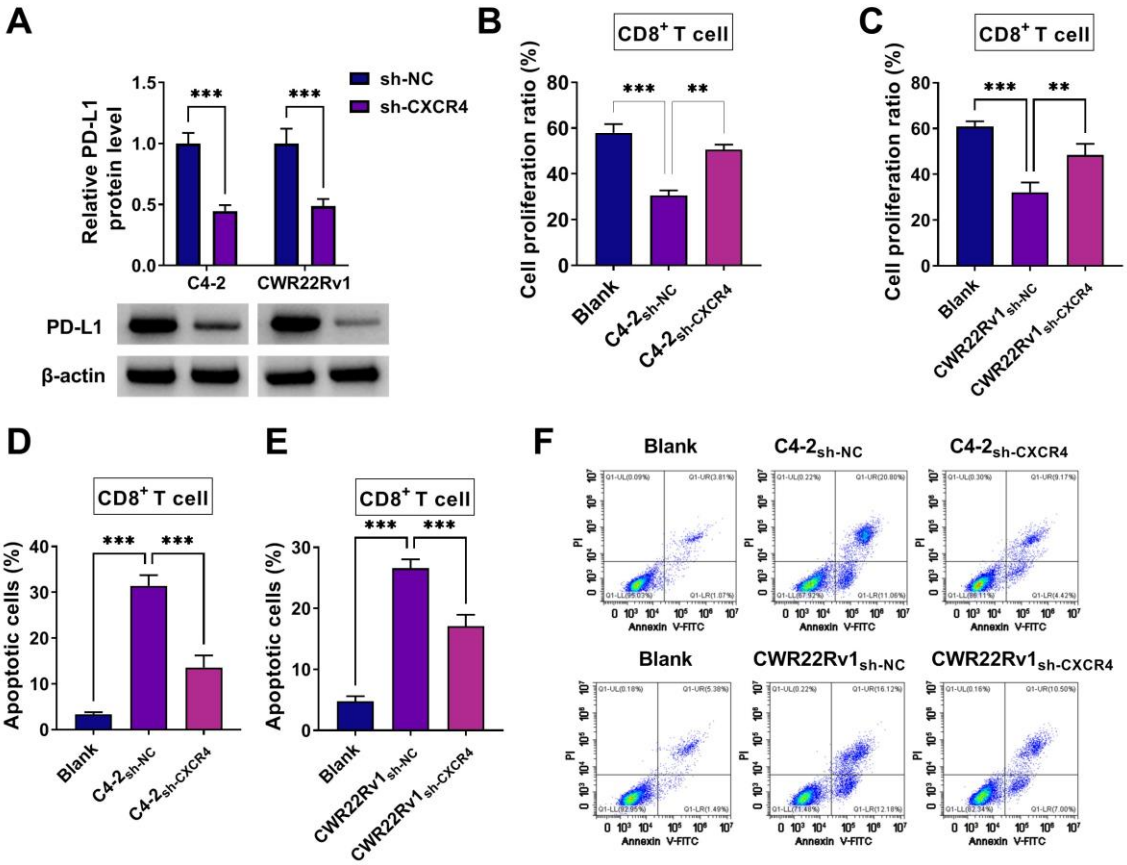

Figure 4. Castration-resistant prostate cancer (CRPC) cells that knocked down C-X-C chemokine receptor type 4 (CXCR4) inhibited cluster of differentiation 8 positive (CD8$^+$) T cell depletion. (A) The expression level of programmed death-ligand 1 (PD-L1) protein in short hairpin RNA targeting CXCR4 (sh-CXCR4) transfected CRPC cells was detected by western blot (WB); (B–C) The proliferative capacity of CD8$^+$ T cells after co-incubation with CRPC cells transfected with sh-CXCR4; (D–F) The proliferative capacity of CD8$^+$ T cells after co-incubation with sh-CXCR4 transfected CRPC cells was detected by flow cytometry (FCM) assay.

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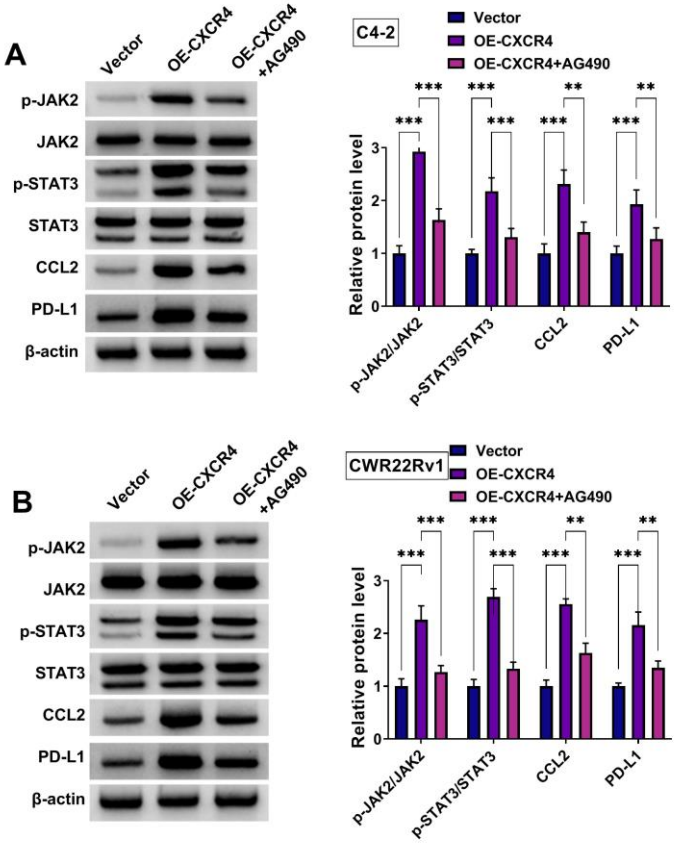

Figure 5. Activation of the Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) pathway by C-X-C chemokine receptor type 4 (CXCR4) enhances the expression of C-C motif chemokine ligand 2 (CCL2) and programmed death-ligand 1 (PD-L1). (A–B) Western blot (WB) analysis of phosphorylated JAK2 (p-JAK2), phosphorylated STAT3 (p-STAT3), PD-L1, and CCL2 protein expression levels in castration-resistant prostate cancer (CRPC) cells overexpressing *CXCR4*, with and without the JAK2-specific inhibitor AG490.

USP10 Promotes CXCR4 Expression Through METTL3

Dataset GSE32269 results showed that METTL3 is upregulated in CRPC tissues (Figure 6A). Predictions from the SRAMP database revealed seven "Very high confidence" methylation sites on *CXCR4* mRNA, suggesting potential methylation modifications of *CXCR4* (Figure 6B). The RBPsuite database predicted binding sites between METTL3 protein and *CXCR4* mRNA (Figure 6C). To verify whether USP10 regulates CXCR4 expression via METTL3, affecting CRPC cell malignancy, macrophage polarization, and immune evasion, we transfected CRPC cells (C4-2 and CWR22Rv1) with sh-NC, sh-USP10, sh-USP10+OE-METTL3, and sh-USP10+OE-CXCR4. WB results showed that knockdown of METTL3 significantly inhibited CXCR4 protein expression (Figure 6D). The GSE32269 dataset also indicated that USP10 is upregulated in CRPC tissues (Figure 6E). Additionally, co-transfection of sh-USP10 and OE-METTL3 overexpression vectors into CRPC cells showed that knock-down of USP10 markedly suppressed the expression of METTL3 and CXCR4 proteins. Elevated expression of METTL3 partially counteracted the inhibitory impact of USP10 knock-down on CXCR4 protein expression (Figures 6F-H). RT-qPCR analysis conducted on 20 CRPC tissues, 20 PCa tissues, and 20 BPH specimens unveiled elevated mRNA levels of USP10 and METTL3 in CRPC tissues relative to both PCa and BPH tissues (Figures 6I–J). Western blot results confirmed that USP10 and METTL3 protein expression was heightened in CRPC tissues in contrast to PCa and BPH tissues (Figure 6K), and both were highly expressed in CRPC cells (Figures 6L–N).

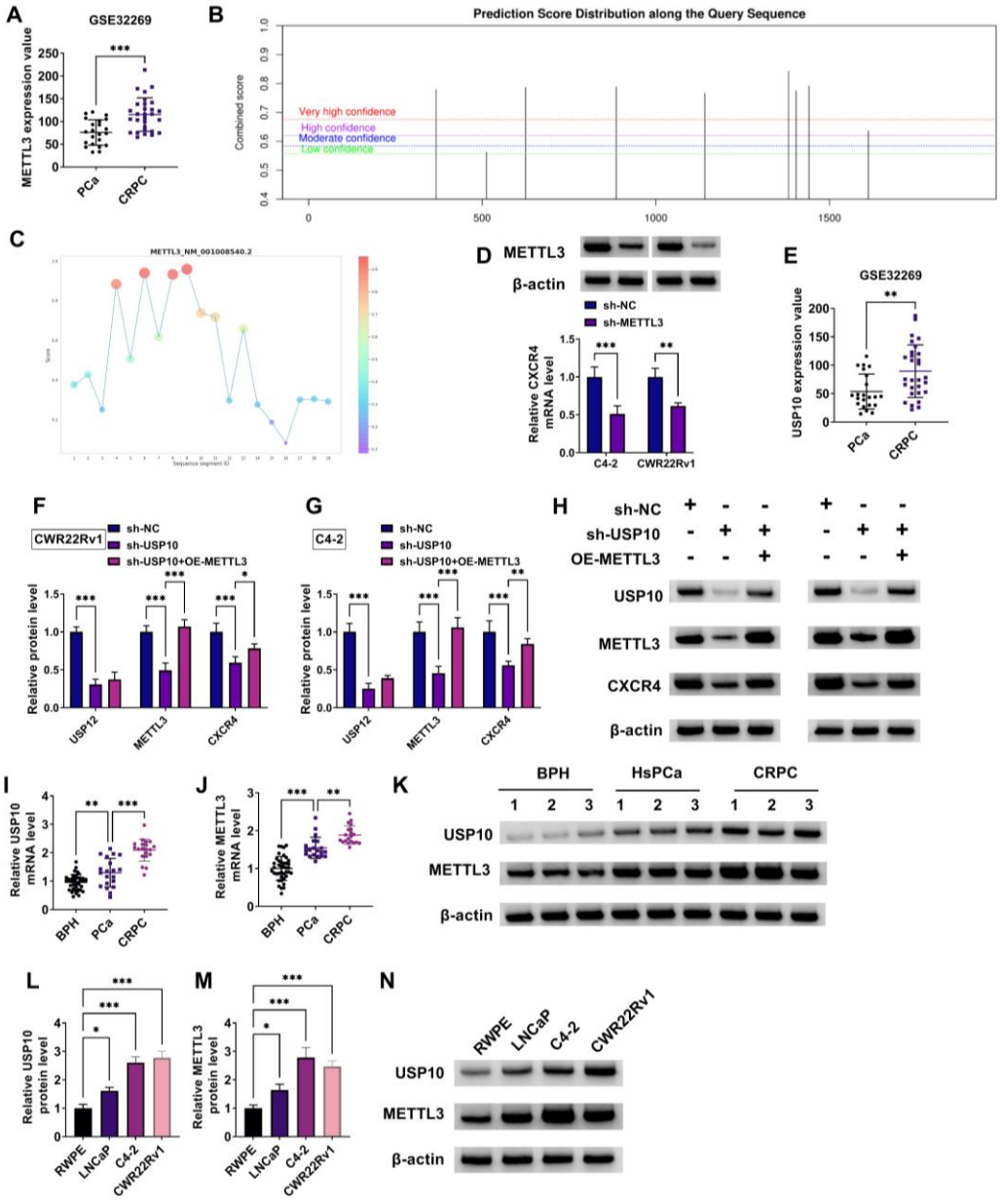

Figure 6. Ubiquitin-specific peptidase 10 (USP10) promotes C-X-C chemokine receptor type 4 (CXCR4) expression through methyltransferase-like 3 (METTL3). (A) The expression level of METTL3 in castration-resistant prostate cancer (CRPC) tissues was analyzed with dataset Gene Expression Omnibus (GSE) 32269; (B) CXCR4 messenger RNA (mRNA) sites were predicted by sequential random adenine methylation prediction (SRAMP) database; (C) The RNA-binding proteins (RBP) suite database predicted the binding sites of METTL3 protein and CXCR4 mRNA; (D) The CXCR4 protein expression level of CRPC cells with METTL3 knocked down was detected by western blot (WB); (E) Dataset GSE32269 was used to analyze the expression level of USP10 in CRPC tissues; (F–H) The levels of expression of USP10, METTL3 and CXCR4 proteins in CRPC cells with short hairpin RNA targeting USP10 (sh-USP10) and overexpression of METTL3 (OE-METTL3) vectors were detected by WB; (I–J) The levels of expression of USP10 and METTL3 in CRPC, prostate cancer (PCa) and benign prostatic hyperplasia (BPH) tissues were detected by reverse transcription quantitative polymerase chain reaction (RT-qPCR); (K) The levels of expression of USP10 and METTL3 in CRPC, PCa and BPH tissues were detected by WB; L-N, The levels of expression of USP10 and METTL3 proteins in CRPC cells were detected by WB.

DISCUSSION

AR is a target for the treatment of advanced PCa, with ADT long regarded as the primary method for managing advanced symptomatic CSPC. Nonetheless, PCa cells have the ability to adjust to low androgen environments by upregulating AR expression and signaling, resulting in the failure of ADT and the emergence of CRPC.[24] Tumor immunotherapy has been a focal point in basic cancer research. Previous studies have indicated that CRPC generally exhibits low immune infiltration and possesses a relatively high immunosuppressive TME, characterized by low levels of T lymphocyte infiltration and activity, additionally, there is elevated expression of several immunosuppressive factors, including PD-1 and PD-L1. Additionally, the infiltration and activity of alternatively activated (M2) macrophages, also known as TAMs, are higher. These TAMs secrete inhibitory cytokines that downregulate the immune response against tumors, classifying CRPC as an immunologically "cold" tumor.[25] Therefore, to overcome AR resistance, it may be necessary to identify suitable immunotherapy strategies.

Chemokines and their receptors play crucial roles in cancer metastasis, with CXCR4 being a chemokine receptor of significant interest.[26] Our research indicates that CXCR4 is upregulated in CRPC tissues and cell lines. Inhibition of CXCR4 can suppress CRPC cell proliferation, migration, invasion, and macrophage M2 polarization while promoting $CD8^+$ T cell exhaustion. Research has demonstrated that CCL2 and PD-L1 in tumor cells act as promoters of macrophage M2 polarization and $CD8^+$ T cell exhaustion, respectively, and their expression can be regulated by the JAK2/STAT3 pathway.[27]

m6A (N6-methyladenosine) modification is a prevalent epigenetic modification on mRNA, playing a critical role in regulating gene expression, mRNA stability, and translation efficiency.[28] METTL3, an m6A methyltransferase, has been reported to promote immune evasion in various cancer cells.[29] Studies have shown that the JNK signaling pathway regulates METTL3-mediated m6A modification of PD-L1 mRNA, thereby promoting bladder cancer immune evasion by resisting the cytotoxicity of $CD8^+$ T cells.[30] METTL3 mediates m6A modification of SCAP mRNA, promoting its translation and reducing the infiltration of granzyme B and interferon-γ-positive $CD8^+$ T cells, thus inhibiting antitumor immune responses and facilitating immune evasion. Targeting METTL3 in combination with anti-PD-1 antibodies can synergistically activate cytotoxic $CD8^+$ T cells and mediate tumor regression in hepatocellular carcinoma.[31] Furthermore, studies have identified DUBs as effective targets for PCa treatment, with specific DUBs regulating AR protein stability and downstream signaling. For example, USP10 can regulate AR transcriptional activity, and USP26 can influence AR activity and stability.[32] Combining bioinformatics predictions and GEO database analyses, we found that USP10 may promote CXCR4 expression by targeting METTL3. Further research indicated that CXCR4 activates the JAK2/STAT3 pathway, promoting CCL2 expression to facilitate macrophage M2 polarization and increasing PD-L1 expression to induce T-cell exhaustion. In conclusion, our study demonstrates that USP10 promotes CXCR4 expression through METTL3, activating the JAK2/STAT3 pathway to facilitate macrophage M2 polarization by upregulating CCL2 expression and enhancing PD-L1 expression contributes to T-cell exhaustion. Therefore, the USP10/METTL3/CXCR4 axis promotes the malignant progression of CRPC cells, M2 polarization of macrophages, and immune escape.

Overall, our investigation highlights the involvement of the USP10/METTL3/CXCR4 axis in driving malignant progression, macrophage polarization, and immune escape of CRPC. Furthermore, it identifies CXCR4 as a promising therapeutic target for CRPC. These findings are significant for the practical application and theoretical exploration of developing new diagnostic and prognostic biomarkers for CRPC.

STATEMENT OF ETHICS

This article does not contain any studies with human participants or animals performed by any of the authors.

FUNDING

This study did not receive any funding in any form.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request

AI ASSISTANCE DISCLOSURE

Not applicable.

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