ICAM1 promotes the invasion and metastasis of Epstein–Barr virus (EBV) positive nasopharyngeal carcinoma (NPC) cells by regulating the CCR7-JAK3/STAT3 axis to modulate PD-L1 expression

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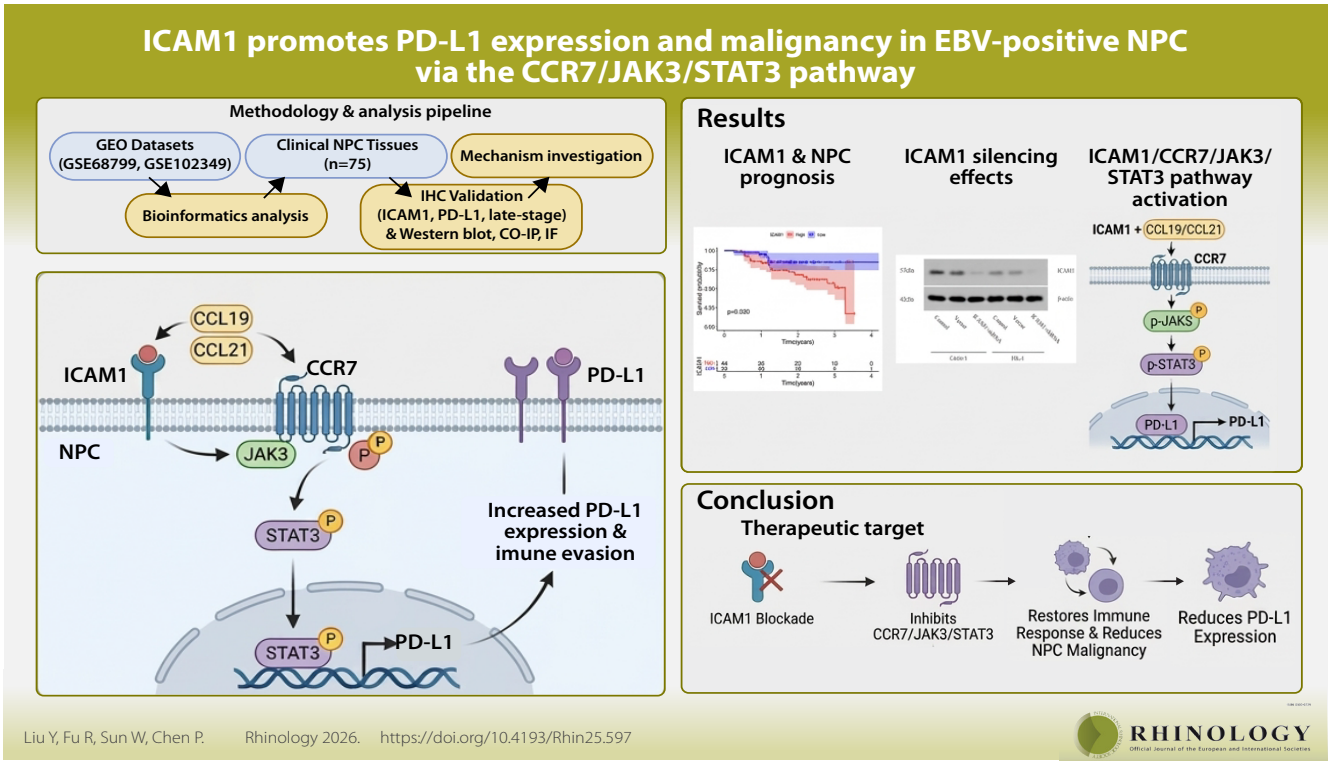

Abstract

Background: In patients with locally advanced, metastatic, or recurrent nasopharyngeal carcinoma (NPC), immunotherapy, especially with programmed cell death ligand 1 (PD-L1) inhibitors, is increasingly being applied for clinical treatment and has shown promising results. Intercellular adhesion molecule 1 (ICAM1) is closely associated with the occurrence and progression of NPC induced by Epstein–Barr virus (EBV) infection and is related to the high expression of PD-L1 and immune suppression in tumour tissues. However, the specific mechanisms are unclear.

Methodology: We used two GEO datasets, GSE68799 and GSE102349, for bioinformatics analysis and performed immunohistochemistry (IHC) on 75 clinical NPC tissue samples. The results from both methods were combined to validate the relationships among ICAM, PD-L1, and patients with late-stage EBV-positive NPC. To elucidate the underlying mechanism, we used Western blotting, coimmunoprecipitation (Co-IP), and immunofluorescence (IF) to investigate the role of ICAM1 in the promotion of NPC cell malignancy.

Results: We found that ICAM1 was highly expressed in EBV-positive NPC tissues and was associated with poor prognosis. Silencing ICAM1 expression decreased the malignant behaviour of NPC cells and PD-L1 expression. The mechanistic study revealed that ICAM1 increased PD-L1 expression, especially at the cell membrane, by activating the JAK3/STAT3 signalling pathway through interactions with CCR7 and its ligands CCL19 and CCL21.

Conclusion: The results of this study indicate that ICAM1 may serve as a therapeutic target for patients with recurrent, metastatic EBV-positive NPC.

Key words: CCR7, ICAM1, JAK3/STAT3 signalling pathway, NPC, PD-L1

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Introduction

Malignant nasopharyngeal carcinoma (NPC) tumours originate from the nasopharynx epithelium. The global annual incidence of NPC is relatively low, typically less than 1/100,000 [1]. However, in southern China and southeastern Asia, the incidence of NPC has increased significantly, especially in southern China (such as Guangdong and Fujian provinces), where the incidence rate of NPC has reached approximately 20/100,000 [2]. EBV infection is strongly correlated with the onset of NPC, and EBV DNA or proteins can be detected in approximately 95% of NPC samples worldwide [1]. Synchronous radiotherapy and chemotherapy are currently the standard treatment regimen for NPC, but immunotherapy is becoming more effective for patients with locally advanced, metastatic and recurrent NPC.

Programmed cell death ligand 1 (PD-L1) is a ligand for programmed cell death protein 1 (PD-1), also known as CD274. PD-L1 is a transmembrane protein primarily expressed on the cell membrane of tumour cells, stromal cells, or tumour-associated macrophages (TAMs) [3]. Studies have shown that the PD-L1 positivity rate in NPC cells is greater than 60%, and many infiltrating T lymphocytes are detected around the cancer nest [4]. PD-L1 expressed by NPC cells can suppress the body's immune response, which allows these cells to escape immune surveillance and be killed by the immune system [5]. Studies have shown that PD-L1 is significantly expressed in NPC tissues and is positively correlated with EBV infection. EBV protein products can promote immune escape by regulating the expression of PD-L1. However, the specific mechanisms and potential targets through which the EBV-dependent promotion of PD-L1 expression induces immune escape are not yet clear.

Intercellular adhesion molecule-1 (ICAM1), also known as CD54, is a transmembrane glycoprotein found on antigen-presenting cells (APCs). It is highly expressed in EBV-positive NPC tissues and is associated with poor patient prognosis [6, 7]. Changes in the level of ICAM1 on the surface of tumour cells affect the killing sensitivity of immune effector cells in tumour tissue [8]. Inhibiting ICAM1 can promote the migration of tumour T cells to lymph nodes and increase immune killing sensitivity [9, 10].

CCR7 was one of the first chemokine receptors (CCRs) discovered in EBV-infected B cells. CCR7 is involved in one of the EBV-related protein pathways and is signature proteins expressed mainly on the surface of immature T cells, B cells and mature dendritic cells (DCs). We found that CCR7 expression is positively correlated with lymphatic infiltration and lymph node metastasis in gastric cancer patients. Tumour cells expressing CCR7 move to lymph nodes under the chemotaxis of their ligand CCL19/CCL21, promoting tumour cell metastasis [11]. Therefore, studying the role of CCR7 and its ligands may help us elucidate the mechanism through which ICAM1 influences PD-L1 expression.

The Janus kinase/signal transducer and activator of transcription (JAK/STAT) signalling pathway is a widely expressed intracellular signalling pathway involved in biological processes such as cell proliferation, differentiation, apoptosis, and immune regulation. Several studies have suggested that the JAK/STAT signalling pathway plays a significant role in PD-L1 expression and the response to anti-PD-L1 treatment [12].

In this study, we conducted an in-depth investigation into the mechanism by which ICAM1 influences PD-L1 expression in NPC cells and elucidated its molecular role in promoting the malignant progression of EBV-positive NPC.

Materials and methods

Patient samples

In this study, data were collected from NPC patients who underwent surgical treatment at the First Affiliated Hospital of Dalian Medical University from January 2010 to January 2022. The inclusion criteria were patients who had never received prior anticancer treatment and were diagnosed with NPC through pathological immunohistochemistry; NPC patients with other malignant tumours or severe systemic diseases were excluded. This study was approved by the First Affiliated Hospital of Dalian Medical University Medical Ethics Committee (No. PJ-KS-KY-2025-471). All patients provided written informed consent prior to surgery. In total, 75 NPC tissue samples were collected and identified as positive or negative for EBV infection by detecting EBER. Immunohistochemical detection was used to compare the expression levels of PD-L1 in EBV-positive and EBV-negative NPC tissues. The basic clinical information and PD-L1 expression levels of all the patients are presented in Table 1.

Bioinformatic enrichment analysis

Two datasets from the GEO (Gene Expression Omnibus) database (GSE68799 and GSE102349) that met the requirements for this study were selected for analysis. Among them, GSE68799 was collected from patients in Shanghai, China, and includes mRNA information from 42 Chinese NPC patients. GSE102349 includes patients from the United States and contains whole-genome data from 113 fresh, untreated undifferentiated NPC tumours. Gene expression data for GSE68799 and GSE102349 were organized, and those from tumour samples only were retained. The expression levels of the genes encoding ICAM1, PD-1 and PD-L1 were obtained. Among them, the gene encoding PD1 is named PDCD1, and the gene encoding PD-L1 is named CD274. After extraction, correlation analysis was performed using the R language cor.R. The survival data of GSE102349 were collected, and survival analysis was performed using the program Survival_best_cutoff.R. The cut-off value was calculated to determine the expression value that best fit the cut-off, and the samples were divided into an ICAM1 high-expression group and a low-expression group, after which a Kaplan-Meier survival curve was constructed. Moreover, the KEGG bioinformatics database

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Table 1. The relationship between PD-L1 expression and clinicopathological characteristics in NPC tissues.

Characteristics	Cases	PD-L1 expression		p-value
		High	Low	
Gender				
Male	51	39	12	0.999
Female	24	18	6	
Age (years)				
>50	47	36	11	0.999
≤50	28	21	7	
Smoking history				
Yes	41	29	12	0.286
No	34	28	6	
EBV infection				
Yes	51	43	8	0.02*
No	24	14	10	
TNM stage (AJCC stage)				
I - III Stages	62	46	16	0.72
IV Stage	13	11	2	
Setting (Local recurrent or Distance metastasis)				
Local recurrence	13	12	1	0.49
Distance metastasis (lung, bone, liver, cervical LN, skin)	10	9	1	
Initial treatment				
PF + RT → PF	18	13	5	0.683
TPS → CDDP + RT	23	20	3	
PCC → CDDP + RT	22	18	4	
Other treatment options	12	10	2	
Pathological classification				
Differentiated non-keratinizing	15	10	5	0.31
Undifferentiated non-keratinizing	60	52	13	
3-year survival rates	68/75 (90.67%)	51/57 (89.47%)	17/18 (94.4%)	

The expression of PD-L1 is not related to the patient's gender, age, smoking, TNM stages, or pathological classification ($p>0.05$), but is related to staging and EBV infection and statistically significant ($p<0.05$). The efficacy of induction chemotherapy and concurrent chemoradiotherapy is not related to PD-L1 expression. TPS: docetaxel + cisplatin + S-1, PF: cisplatin + 5-FU, PCC: paclitaxel + carboplatin + cetuximab, CDDP: isplatin, RT: radiotherapy.

(https://www.kegg.jp/) was used to analyse and predict EBV cell pathways.

NPC cell culture

Two NPC cell lines, C666-1 (poorly differentiated and EBV-positive) and HK-1 (well differentiated and EBV-negative), were purchased from the Chinese Academy of Sciences Cell Bank. All the cells were maintained in 1640 medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% foetal bovine serum (FBS; Gibco, Thermo Fisher Scientific), 100 U/mL penicillin G, and 100 µg/mL streptomycin (Gibco) at 37°C with 5% CO_2/95% air.

Cell transfection

Small interfering RNAs (siRNAs) were purchased from GenePharma (GenePharma Co. Ltd., Shanghai, P. R. China). The ICAM1-siRNA sequence of the target region was 5'-CAA GAA AUA CAG ACU ACA ATT-3'. The CCR7-siRNA sequence of the target region was 5'-GGC UGG UCG UGU UGA CCU ATT-3'. The NC-siRNA sequence of the target region was 5'-UUC UCC GAA CGU UGC ACG UTT-3'. Cells were transfected with siRNA using Lipofectamine 3000 (Thermo Fisher Scientific; Invitrogen, Carlsbad, CA, USA) in medium without antibiotics according to the manufacturer's instructions. The silencing efficiency was verified by Western blotting.

The overexpression lentiviral vector encoding human ICAM1 and CCR7 cDNA (OE) and an empty vector (EV) were generated by GenePharma (GenePharma Co. Ltd., Shanghai, P. R. China). ICAM1-shRNA lentivirus, CCR7-shRNA lentivirus and negative control lentivirus were generated by GenePharma (GenePharma

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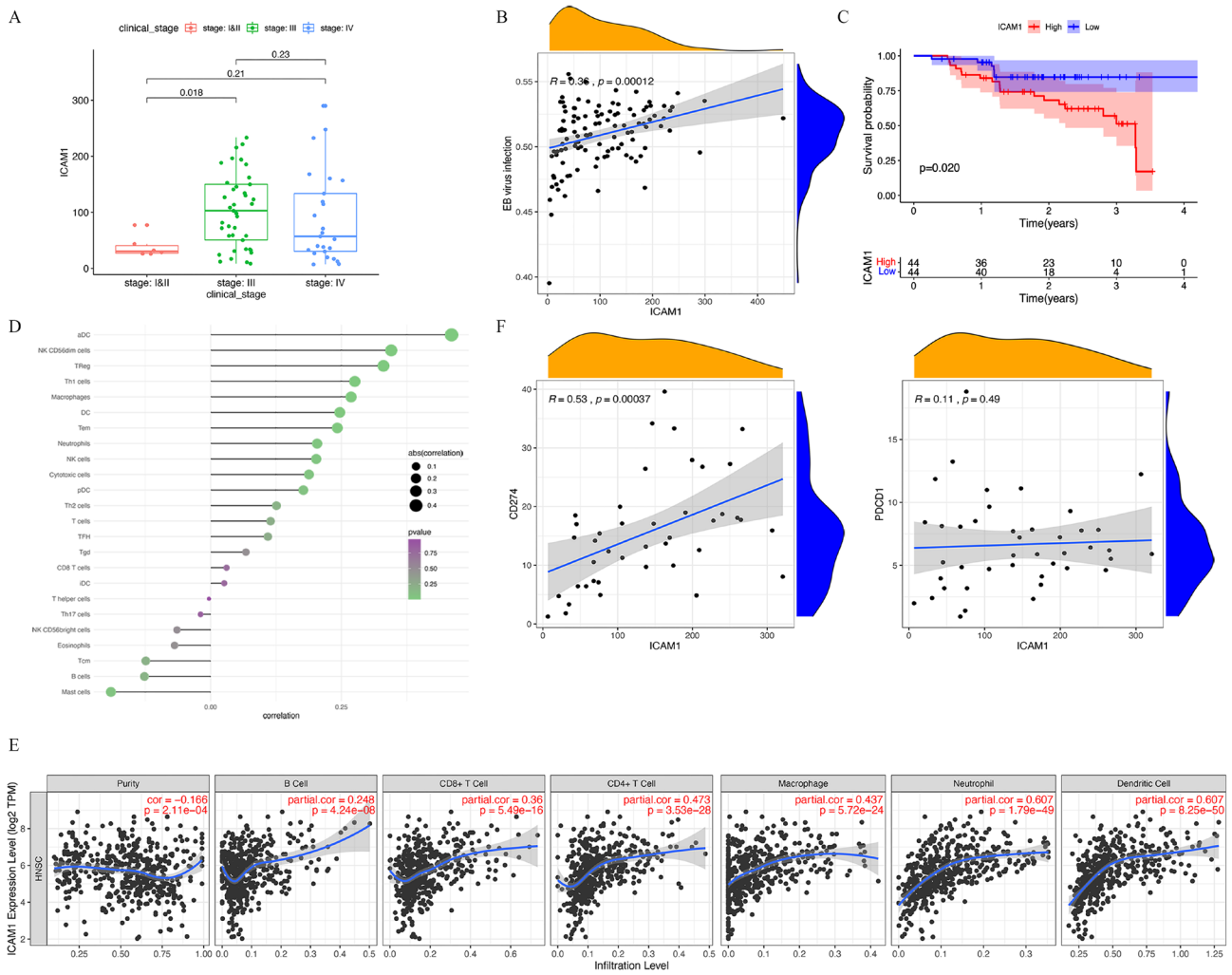

Figure 1. A) Analysis of gene expression data in GSE68799 and GSE102349 revealed that ICAM1 expression was significantly higher in NPC tissues from patients with stage III disease than in NPC tissues from patients with stage I-II disease. B) ICAM1 expression was significantly higher in EBV-positive NPC tissue. C) ICAM1 expression was positively correlated with poor prognosis. D) Relationships between ICAM1 expression levels and infiltrating immune cells surrounding the tumour. E) ICAM1 expression was positively correlated with PD-L1 expression but not with PD-1 expression. F) ICAM1 is positively associated with B cells, CD4+ T cells, CD8+ T cells, macrophages, neutrophils and dendritic cells.

Co. Ltd., Shanghai, P. R. China) for stable silencing. The sequences were as follows: Lv-Homo-CCR7-shRNA: 5'-ccg CCC TAT CAT GTA CTC CAT CAT ttc aag aga ATG ATG GAG TAC ATG ATA GGG ttt tt-3'; Lv-Homo-ICAM1-shRNA: 5'-ccg CGG CTG ACG TGT GCA GTA ATA ttc aag aga TAT TAC TGC ACA CGT CAG CCG ttt tts-3'; and shNC: 5'-ccg TTC TCC GAA CGT GTC ACG Ttt caa gag aAC GTG ACA CGT TCG GAG Aat tttt-3'. The overexpression and silencing efficiencies were verified by Western blotting.

Wound healing and Transwell assays

For the wound healing assay, after transfection for 24 h, C666-1 and HK-1 cells were seeded in a 6-well plate (Millipore, Burlington, MA, USA). After 24 hours, the cells had grown to nearly 90% confluence, and the cell layer was scratched with a 200 µL pipette tip and the medium containing 10% FBS was replaced with serum-free medium. Images of the cells were captured at 0 h, 6 h, 12 h and 24 h.

For the Transwell invasion assay, after transfection for 24 h, 1×10^4 C666-1 and HK-1 cells were seeded in 200 µL serum-free medium in the upper chamber of a 24-well Transwell chamber (Millipore, Burlington, MA, USA) according to the manufacturer's protocol. For the migration assay, 5×10^4 cells in 400 µL of serum-free RPMI-1640 medium were loaded into the upper chambers, while the lower chambers were supplemented with 0.6 mL of RPMI-1640 medium supplemented with 10% FBS. For the invasion assay, 5×10^4 cells were incubated in the upper chambers with membranes coated with Matrigel (1:6 dilution; BD Biosciences, San Jose CA, USA). After 24 h of incubation, the insert plates were rinsed with 1× PBS, and the upper surfaces of the membranes were scraped to remove cells. After being fixed

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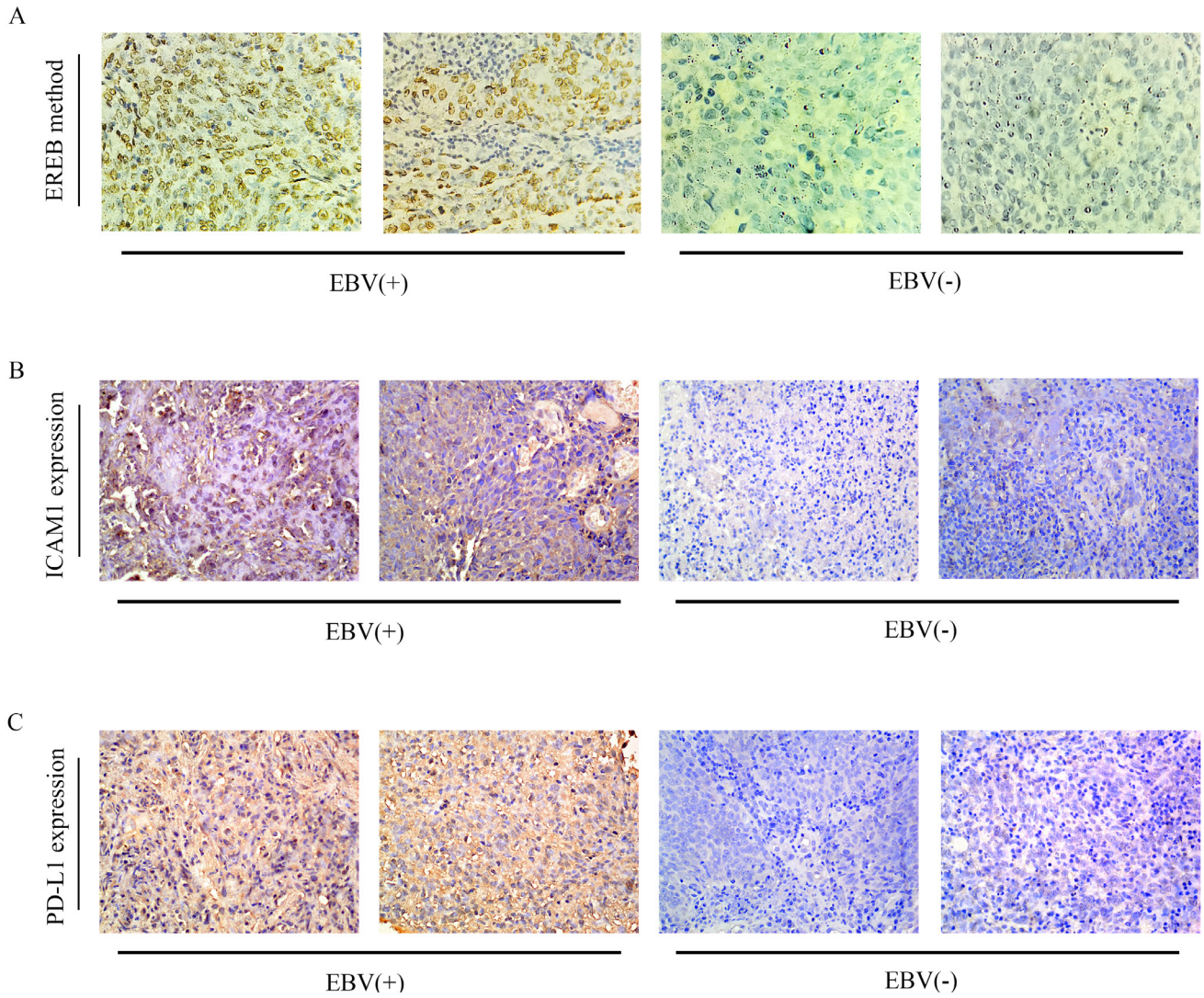

Figure 2. A) EBER in situ hybridization was applied to identify EBV-DNA-positive and EBV-DNA-negative NPC tissue. B) Immunohistochemistry was used to detect the expression of ICAM1. C. Immunohistochemistry was used to detect the expression of PD-L1.

with 4% methanol for 15 min, the cells on the underside of the membrane were stained with crystal violet at room temperature and counted under a microscope.

Western blotting

Forty micrograms of protein from each cell line were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The antibodies used for intracellular and cell-surface staining were as follows: anti-ICAM1 (1:1,000; Proteintech, Rosemont, IL, USA), anti-CCR7 (1:1,000; Proteintech), anti-CD274-fluorescein isothiocyanate (FITC) mAb (1:100; Abcam, Cambridge, UK), anti-CCL19 (1:1, Proteintech), anti-CCL21 (1:500; Proteintech), anti-JAK3 (1:500; Proteintech), anti-STAT3 (1:500; Proteintech) and anti-β-actin (1:2,000; Proteintech). The proteins were detected using enhanced chemiluminescence reagent (Santa Cruz Biotechnology, Dallas, TX, USA). Western blot signals were quantified using an Amersham Imager 600 (GE Healthcare, Little Chalfont, UK), and band signals are expressed as relative amounts of protein compared with those of β-actin.

Immunohistochemistry (IHC) assay

After deparaffinization with xylene and dehydration with a serial ethanol gradient, antigen retrieval was performed by heating the slides in citrate buffer (pH 6.0) for 7 min on high heat. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide. The samples were incubated overnight at 4°C with anti-ICAM1 (1:100; Sigma, St. Louis, Missouri, USA). Slides were stained with 3,3′-diaminobenzidine and haematoxylin, dehydrated through an ethanol gradient, and mounted. The percent area with positive protein expression was evaluated by ImageJ software (1.48v; Wayne Rasband National Institutes of Health, Bethesda, MD, USA) (area ≥50%: high expression; area <50%: low expression).

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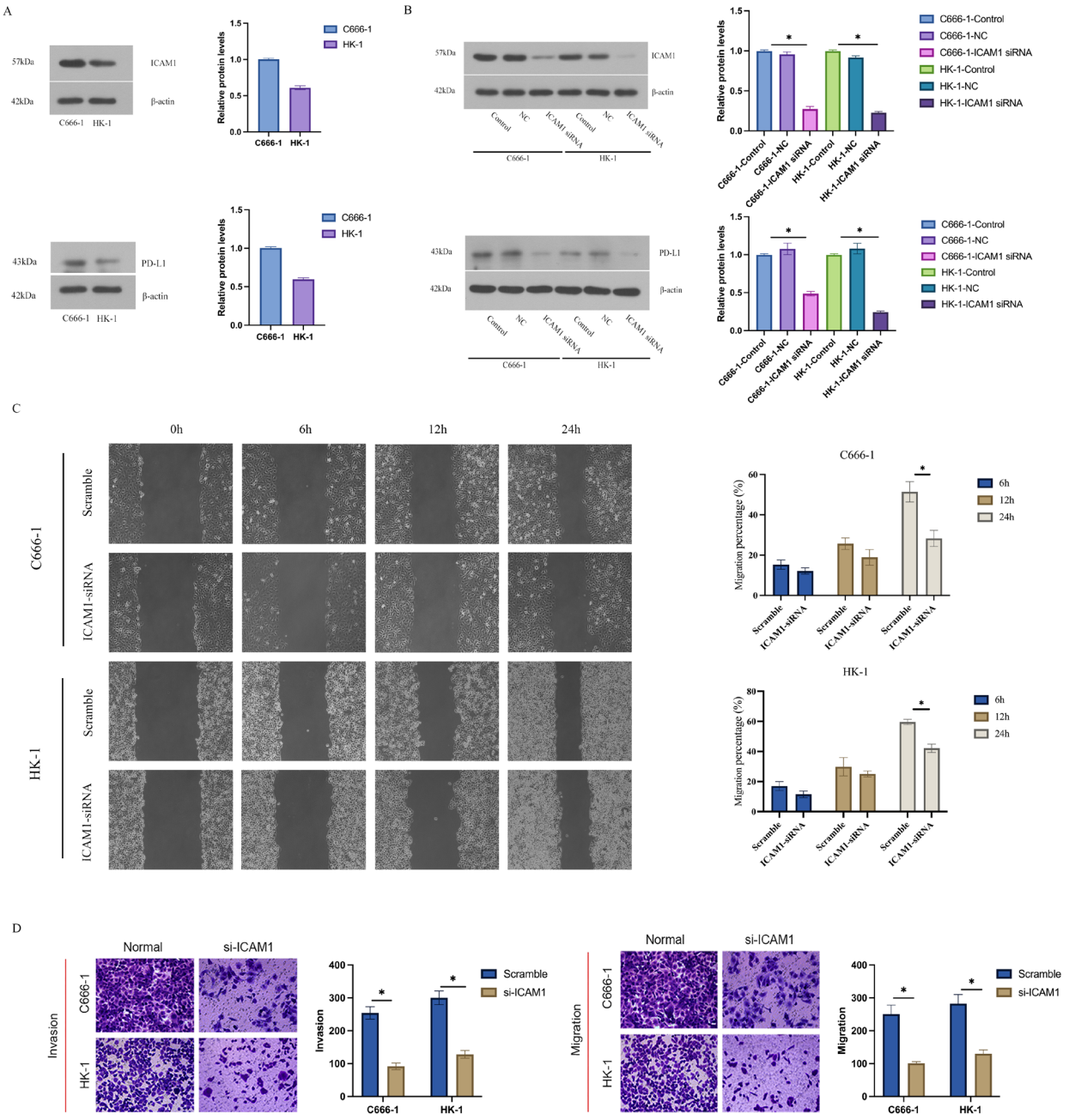

Figure 3. A) The expression levels of both ICAM1 and PD-L1 were slightly greater in C666-1 cells than in HK-1 cells. B) After silencing ICAM1, the expression levels of both ICAM1 and PD-L1 decreased. C) After silencing ICAM1 in C666-1 and HK-1 cells, the migration ability significantly decreased. D) After silencing ICAM1 in C666-1 and HK-1 cells, the invasion ability significantly decreased. (Scramble: untreated group; si-ICAM1: cells treated with ICAM1-siRNA) (*p<0.05).

Coimmunoprecipitation (Co-IP)

Briefly, C666-1 and HK-1 cells were collected and lysed in IP lysis buffer supplemented with PMSF (protease inhibitor; ST505; Beyotime, China). For Co-IP, the lysates of 1×10^7 C666-1 and HK-1 cells were immunoprecipitated with IP buffer containing IP antibody-coupled agarose beads, and protein-protein complexes were subsequently subjected to Western blotting with anti-ICAM1 (1:1,000; Proteintech) and anti-CCR7 (1:5,00; Proteintech) antibodies. IgG was used as a negative control. For additional details, please refer to the Pierce™ Immunoprecipitation Kit protocol (#26149; Thermo Fisher Scientific).

Immunofluorescence

Cultured NPC cells were placed on appropriate glass slides and allowed to adhere. The cells were fixed for approximately 15 min with 4% formaldehyde and then washed three times with

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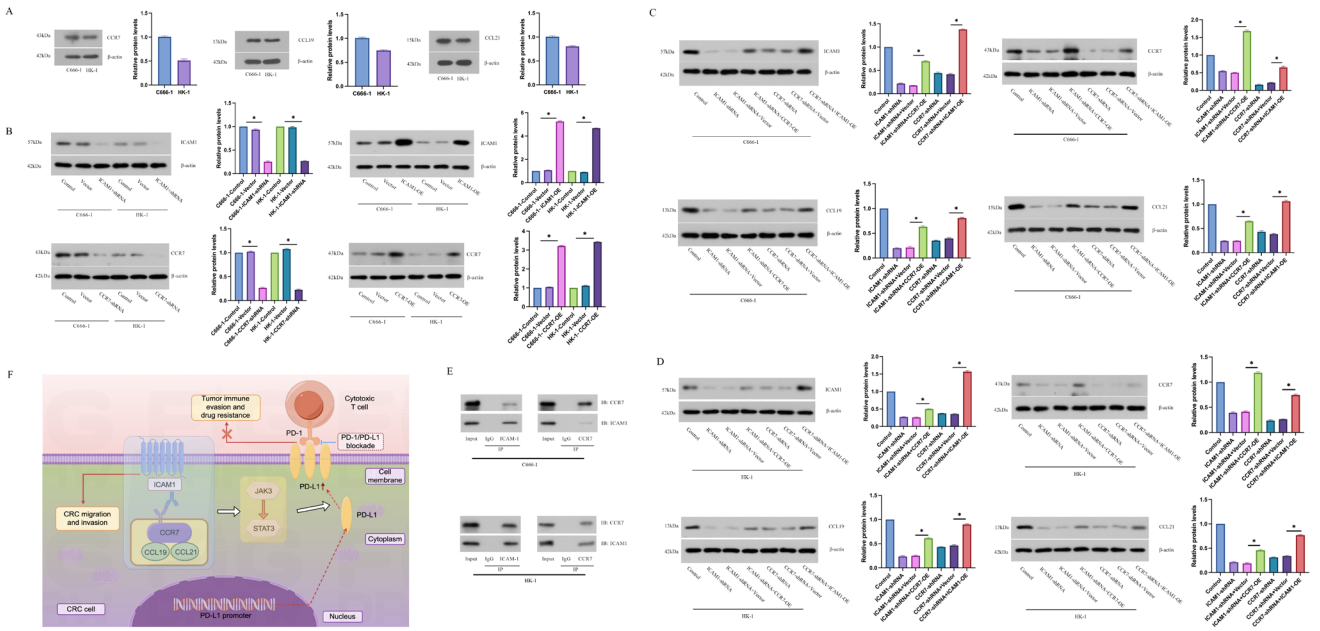

Figure 4. A) Western blot analysis revealed that the expression levels of CCR7, CCL19 and CCL21 did not significantly differ between C666-1 and HK-1 cells. B) Western blot analysis verified the stable silencing and overexpression efficiency of ICAM1 and CCR7. C-D) Western blot analysis verified the regulatory effect of ICAM1 on the expression of CCR7 and its ligands CCL19 and CCL21 in C666-1 and HK-1 cells. E) Co-IP verified the direct physical interaction between ICAM1 and CCR7. F) Diagram illustrating the interactions among proteins.

PBS. The slides were blocked with 5% goat serum and anti-PD-L1 immunofluorescence antibodies (1:500; Proteintech) and incubated overnight at 4°C. The cells were subsequently washed to remove unbound primary antibodies and incubated with secondary antibody (1:1000; Proteintech) at 37°C for 1 hour in the dark. Afterwards, the cells were stained with 4',6-diamidino-2-phenylindole (DAPI) to label the cell nuclei for approximately 5 min, after which the excess DAPI was removed by washing. Cells were analysed with a laser confocal fluorescence microscope, and the distribution of proteins in the NPC cells was record.

Statistical analysis

Representative data from three separate experiments are shown as the mean±standard deviation. For bioinformatics analysis, statistical analysis was performed according to the default parameters of the corresponding software. Statistical differences between groups were compared using chi-square tests, Fisher's tests, and t tests; P values <0.05 were considered to indicate statistical significance. P values <0.01 were considered to indicate obvious statistical significance.

Results

Bioinformatic enrichment analyses

Analysis of the gene expression data in datasets GSE68799 and GSE102349 revealed that ICAM1 expression was significantly higher in NPC tissues from patients with stage III disease than in NPC tissues from patients with stage I-II disease (Figure 1A) and correlated with EBV infection (Figure 1B) and poor prognosis (Figure 1C). The interaction between ICAM1 and infiltrating immune cells was also analysed (Figure 1D). ICAM1 was positively associated with B cells, $CD4^+$ T cells, $CD8^+$ T cells, macrophages, neutrophils and dendritic cells (Figure 1E). Further analysis revealed that ICAM1 expression was positively correlated with PD-L1 expression but not correlated with PD-1 expression (Figure 1F).

Effect of EBV infection on the expression of ICAM1 and PD-L1 in NPC tissues

Tissue samples were collected from 75 NPC patients at the First Affiliated Hospital of Dalian Medical University, and EBER in situ hybridization was used to identify 51 patients who were positive or weakly positive for EBV-DNA expression and 24 patients who were negative for EBV-DNA expression (Figure 2A). Immunohistochemistry (IHC) was used to detect the expression of ICAM1 and PD-L1 in these NPC patients, and the expression levels of ICAM1 (Figure 2B) and PD-L1 (Figure 2C) were significantly greater in the 51 EBV-infected patients than in the 24 EBV-negative patients. The detailed clinical pathological information and PD-L1 expression levels of all the patients are presented in Table 1.

ICAM1 promotes the migration and invasion of NPC cells and regulates the expression level of PD-L1

We first compared the expression levels of ICAM1 and PD-L1 in C666-1 and HK-1 cells. The results revealed that the expression

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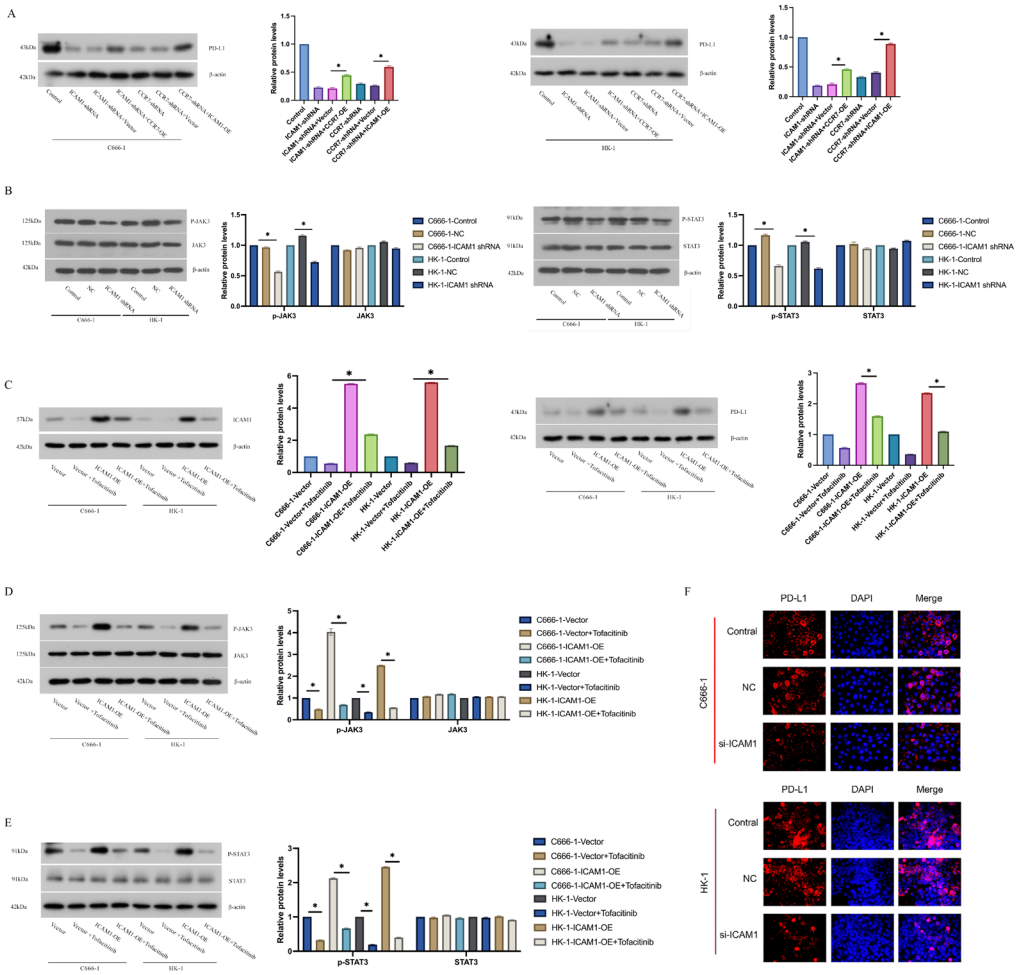

Figure 5. A) Western blot analysis revealed that when ICAM1 and CCR7 were silenced, the expression of PD-L1 decreased; however, when ICAM1 was overexpressed in CCR7-silenced NPC cells or when CCR7 was overexpressed in ICAM1-silenced NPC cells, the expression of PD-L1 increased, and the regulatory effect of ICAM1 was more obvious. B) Western blot analysis revealed that after treatment with ICAM1-shRNA, the expression levels of phospho-JAK3 and phospho-STAT3 decreased. C) After C666-1 and HK-1 cells were treated with ICAM1 overexpression lentivirus, the expression of PD-L1 clearly increased, but this effect was reversed by the JAK3/STAT3 pathway inhibitor tofacitinib. D-E) When ICAM1 was overexpressed, the expression levels of pho-JAK3 and pho-STAT3 were clearly upregulated, but this effect was reversed by the JAK3/STAT3 pathway inhibitor tofacitinib. F) Immunofluorescence analysis revealed that ICAM1 promotes the expression of PD-L1 on the C666-1 cell and HK-1 cell membrane. Red staining represents PD-L1 expression, and blue staining indicates the nuclei (DAPI) (magnification, 400×).

levels of both ICAM1 and PD-L1 were slightly greater in C666-1 cells than in HK-1 cells. Although there was no significant difference between the two, it was still possible to observe trends of higher ICAM1 and PD-L1 expression in NPC cells that are positive for EBV infection (Figure 3A). ICAM1 was subsequently silenced in C666-1 and HK-1 cells with ICAM1-siRNA, and the silencing efficiency was verified by Western blotting (Figure 3B). After silencing of ICAM1, the expression levels of both ICAM1 and PD-L1 decreased (Figure 3B), indicating that ICAM1 may increase the expression of PD-L1. By using wound healing and Transwell assays, we also revealed that the migration and invasion capacities of these cells clearly decreased after silencing ICAM1 (Figure 3C–3D), indicating that ICAM1 promotes malignant behaviours in NPC cells.

ICAM1 affects the expression of PD-L1 by interacting with CCR7 and its ligands CCL19 and CCL21

To explore how ICAM1 affects PD-L1 expression, we stably silenced ICAM1 expression in C666-1 and HK-1 cells with an ICAM1-shRNA lentivirus and a CCR7-shRNA lentivirus and overexpressed ICAM1 and CCR7 with an overexpression lentivirus. First, Western blot analysis revealed that the expression levels of CCR7, CCL19 and CCL21 did not significantly differ between C666-1 and HK-1 cells (Figure 4A). Second, Western blot analysis revealed a significant decrease in the expression of CCR7 and its ligands CCL19 and CCL21 after ICAM1-shRNA treatment, whereas these effects were partially reversed after CCR7 was overexpressed. In contrast, the expression of ICAM1, CCL19 and CCL21 was also significantly reduced after treatment with CCR7-

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shRNA, but these effects were significantly reversed after ICAM1 was overexpressed (Figure 4B–D). Furthermore, using Co-IP, we validated a direct physical interaction between ICAM1 and CCR7 (Figure 4E). The above results indicated that ICAM1 and CCR7 interact with each other and jointly influence the downstream ligands CCL19 and CCL21, but ICAM1 plays a more dominant role. Therefore, we speculate that ICAM1 affects PD-L1 expression by interacting with CCR7. A diagram illustrating the interactions among these proteins is shown in Figure 4F.

ICAM1 affects the expression of PD-L1 by regulating the JAK3/STAT3 signalling pathway

Western blotting revealed that after silencing ICAM1 and CCR7, the expression of PD-L1 decreased; however, after ICAM1 was overexpressed in CCR7-silenced NPC cells or CCR7 was overexpressed in ICAM1-silenced NPC cells, the expression of PD-L1 increased, and the regulatory effect of ICAM1 was more obvious (Figure 5A). The results revealed that CCR7 expression was positively correlated with PD-L1 and ICAM1 expression levels in C666-1 and HK-1 cells.

Western blotting was also used to verify the relationship between ICAM1 and the JAK3/STAT3 signalling pathway. We treated C666-1 and HK-1 cells with ICAM1-shRNA lentivirus and found that the phosphorylation levels of JAK3 and STAT3 decreased, indicating that ICAM1 can activate the JAK3/STAT3 pathway (Figure 5B). After ICAM1 was overexpressed, the phosphorylation levels of JAK3 and STAT3 clearly increased. However, after treatment with the JAK3/STAT3 pathway inhibitor tofacitinib, the activating role of ICAM1 was abolished (Figure 5D–E). Next, when ICAM1 was overexpressed in C666-1 and HK-1 cells, the expression of PD-L1 clearly increased, but this effect was reversed by the JAK3/STAT3 pathway inhibitor tofacitinib (Figure 5C). On the basis of the above results, we concluded that ICAM1 plays an important regulatory role in the JAK3/STAT3 pathway to influence the expression of PD-L1. We also validated the effects of ICAM1 on PD-L1 expression and colocalization with PD-1 on the surface of HK-1 cells by IF. These results suggest that ICAM1 increased PD-L1 expression on the NPC cell membrane (Figure 5F).

Discussion

EBV infection is a significant cause of NPC. EBV infection can induce the transformation of nasopharyngeal mucosal cells into cancerous cells, and EBV DNA or related antigens can be detected in the tumour tissue of nearly all patients with nonkeratinizing NPC. Studies have shown that PD-L1 is significantly expressed in NPC tissues and is positively correlated with EBV infection. EBV protein products can promote immune escape by regulating the expression of PD-L1. In our study, we confirmed that EBER was highly expressed in patients with recurrent and late-stage disease and was potentially positively correlated with poor prognosis.

The interaction between PD-L1 and PD-1 is one of the ways in which NPC cells suppress the immune response of the body, thus avoiding detection and killing by the immune system. Several studies have shown that factors in the tumour environment upregulate PD-L1 expression in lung cancer cells, MDSCs, and T cells and inhibit the antitumour immune response by providing an inhibitory signal for T-cell activation [13]. ICAM1 is a transmembrane glycoprotein. Changes in the level of ICAM1 on tumour cells affect the cytotoxic sensitivity of immune effector cells in tumour tissues. Inhibiting ICAM1 can promote the migration of tumour T cells to lymph nodes and increase immune cytotoxic sensitivity [8]. In this study, we revealed that ICAM1 is associated with late-stage disease and poor prognosis in EBV-positive NPC patients and confirmed the role of ICAM1 in promoting the migration and invasion of C666-1 and HK-1 cells, especially in C666-1 cells, which are EBV-positive. In the immune-related analysis of this study, ICAM1 was verified to increase the expression level of PD-L1. Furthermore, immunofluorescence staining revealed that ICAM1 increases PD-L1 expression on NPC cell membranes. These findings suggest that ICAM1 may be involved in the immune evasion of NPC cells.

With respect to the mechanisms affecting the expression levels of PD-L1 in tumour cells, in a breast cancer study, PD-L1 expression in tumour cells was found to be induced by interferon-γ (IFN), whose surface is regulated by the JAK3/STAT3 signalling pathway [14]. Research on prostate cancer has also shown that activation of the JAK3/STAT3 pathway is involved in regulating PD-L1 expression. The combination of JAK3 or STAT3 inhibitors with anti-PD-L1 antibodies has shown more significant effects than anti-PD-L1 antibodies or JAK3/STAT3 inhibitors alone [15, 16]. The JAK3/STAT3 pathway is an important regulator of chemokine receptor signalling. Specifically, different members of the Janus family (JAK1, JAK2, JAK3, and TYK2) have been reported to phosphorylate tyrosine residues, play key roles in cytokine signalling pathways, and regulate the development, proliferation, and differentiation of various cells [17] upon stimulation by different chemokines in different cell types. Targeting the JAK3 signalling pathway has potent immune suppression and anticancer effects. In the response of peripheral blood lymphocytes to CCL19 and CCL21, JAK3 phosphorylation is critical for the homing of T lymphocytes to peripheral lymph nodes [18]. A recent study demonstrated that activated STAT3 binds to the PD-L1 promoter and subsequently promotes PD-L1 transcription [19]. In other head and neck tumours, migration and invasion experiments and immunofluorescence staining revealed that CCL19 promotes the migration and invasion of and F-actin recombination in CCR7-expressing SCCHN cells, in part by activating the JAK3 signalling pathway [18]. Furthermore, lymphocyte proliferation and contact allergy experiments revealed a decrease in DC-mediated T lymphocyte activity in the absence of JAK3, sugges-

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ting that JAK3 plays a critical role in DC maturation, migration and function through the CCR7-mediated signalling pathway [20]. These results indicate that the JAK3 signalling pathway plays an important role in the malignant biological behaviours associated with CCR7-mediated immune regulation.

To further investigate how ICAM1 regulates PD-L1 expression, we performed bioinformatics analysis and found a potential correlation between ICAM1 expression and the PD-L1-associated JAK3/STAT3 pathway. We then first validated the interaction between ICAM1 and CCR7 and its related ligands CCL19 and CCL21 via Western blotting and Co-IP. We also validated the regulatory role of ICAM1 in the JAK3/STAT3 signalling pathway and suggested that ICAM1 influences PD-L1 expression through the CCR7-mediated activity of the JAK3/STAT3 pathway and its associated ligands, CCL19 and CCL21.

Conclusion

EBV protein products can promote immune escape by regulating the expression of PD-L1 through ICAM1, providing clues for understanding potential targets for NPC immunotherapy in EBV-positive tumours.

Acknowledgments

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Author contributions

YL, PC and WS designed the study. YL and RF performed all the experiments. YL and RF wrote the draft. PC and WS corrected the manuscript. All authors read and approved the manuscript.

Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

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References

1. Su ZY, Siak PY, Lwin YY, Cheah SC. Epidemiology of nasopharyngeal carcinoma: current insights and future outlook. Cancer Metastasis Rev. 2024;43(3):919-39.
2. Li QJ, Fang XL, Li YQ, et al. DCAF7 acts as a scaffold to recruit USP10 for G3BP1 deubiquitylation and facilitates chemoresistance and metastasis in nasopharyngeal carcinoma. Adv Sci (Weinh). 2024;11(36):e2403262.
3. Sprooten J, Vanmeerbeek I, Datsi A, et al. Lymph node and tumor-associated PD-L1(+) macrophages antagonize dendritic cell vaccines by suppressing CD8(+) T cells. Cell Rep Med. 2024;5(1):101377.
4. He F, Gong Y, Tao G, t al. Targeting the LMP1-ALIX axis in EBV(+) nasopharyngeal carcinoma inhibits immunosuppressive small extracellular vesicle secretion and boosts anti-tumor immunity. Cancer Commun (Lond). 2024;44(12):1391-413.
5. Ge J, Liu Y, Chen P, et al. FOXA1 enhances antitumor immunity via repressing interferon-induced PD-L1 expression in nasopharyngeal carcinoma. J Immunother Cancer. 2024;12(11).
6. Busson P, Zhang Q, Guillon JM, et al. Elevated expression of ICAM1 (CD54) and minimal expression of LFA3 (CD58) in Epstein-Barr-virus-positive nasopharyngeal carcinoma cells. Int J Cancer. 1992;50(6):863-7.
7. Lin Z, Liu M, Xing W, et al. A near-infrared fluorescence-enhancing plasmonic biosensing microarray identifies soluble PD-L1 and ICAM-1 as predictive checkpoint biomarkers for cancer immunotherapy. Biosens Bioelectron. 2023;240:115633.
8. Qian WJ, Yan JS, Gang XY, et al. Intercellular adhesion molecule-1 (ICAM-1): From molecular functions to clinical applications in cancer investigation. Biochim Biophys Acta Rev Cancer. 2024;1879(6):189187.
9. Sun Y, Yee E, Fujiwara Y, et al. CD93 blockade promotes effector T-cell infiltration and facilitates adoptive cell therapy in solid tumors. J Immunother Cancer. 2025;13(1).
10. Zhang W, Zhong W, Wang B, et al. ICAM-1-mediated adhesion is a prerequisite for exosome-induced T cell suppression. Dev Cell. 2022;57(3):329-43 e7.
11. Xu D, Liu X, Ke S, Guo Y, Zhu C, Cao H. CCL19/CCR7 drives regulatory T cell migration and indicates poor prognosis in gastric cancer. BMC Cancer. 2023;23(1):464.
12. Chen M, Wang S. Preclinical development and clinical studies of targeted JAK/STAT combined Anti-PD-1/PD-L1 therapy. Int Immunopharmacol. 2024;130:111717.
13. Jeong H, Koh J, Kim S, et al. Cell-intrinsic PD-L1 signaling drives immunosuppression by myeloid-derived suppressor cells through IL-6/Jak/Stat3 in PD-L1-high lung cancer. J Immunother Cancer. 2025;13(3).
14. Mittendorf EA, Philips AV, Meric-Bernstam F, et al. PD-L1 expression in triple-negative breast cancer. Cancer Immunol Res. 2014;2(4):361-70.
15. Yin Y, Wang Z, Hu Y, Wang J, Wang YI, Lu Q. Caffeic acid hinders the proliferation and migration through inhibition of IL-6 mediated JAK-STAT-3 signaling axis in human prostate cancer. Oncol Res. 2024;32(12):1881-90.
16. Palicelli A, Croci S, Bisagni A, et al. What do we have to know about PD-L1 expression in prostate cancer? a systematic literature review. Part 3: PD-L1, Intracellular Signaling Pathways and Tumor Microenvironment. Int J Mol Sci. 2021;22(22).
17. Zhang MX, Jing LY, Tan HT, et al. MIAT promotes tumor-infiltrating CD8(+) T-cell exhaustion and malignant progression of renal cell carcinoma via activating JAK3/STAT3 pathway. J Immunother Cancer. 2025;13(7).
18. Zhang Z, Liu F, Li Z, Wang D, Li R, Sun C. Jak3 is involved in CCR7-dependent migration and invasion in metastatic squamous cell carcinoma of the head and neck. Oncol Lett. 2017;13(5):3191-7.
19. Zhang N, Zeng Y, Du W, et al. The EGFR pathway is involved in the regulation of PD-L1 expression via the IL-6/JAK/STAT3 signaling pathway in EGFR-mutated non-small cell lung cancer. Int J Oncol. 2016;49(4):1360-8.
20. Gebauer N, Kunstner A, Ketzer J, et al. Genomic insights into the pathogenesis of Epstein-Barr virus-associated diffuse large B-cell lymphoma by whole-genome and targeted amplicon sequencing. Blood Cancer J. 2021;11(5):102.

Corrected Proof

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