Abstract. The immune checkpoint molecule B7 family member H4 (B7-H4) plays a similar role to programmed death-ligand 1 in tumor immune evasion by regulating T-cell-mediated immune responses. However, besides the role in T-cell immunity, B7-H4 also affects tumor cell biology by promoting tumor cell proliferation, metastasis and angiogenesis. In order to explore the effect of B7-H4 on tumor cell biology, it is necessary to investigate the gene expression profile when B7-H4 is overexpressed. In the present study, 786-O cells were transfected to stably express B7-H4. A microarray technique was subsequently used to screen B7-H4-related differentially expressed genes (DEGs) in B7-H4/786-O cells compared with negative control (NC)/786-O cells. The protein expression of the upregulated DEGs, including non-metastatic cells 5, NME/NM23 family member 5 (NME5), membrane metalloendopeptidase (MME), vascular non-inflammatory molecule 1 (VNN1), matrix metalloproteinase (MMP) 7, tumor necrosis factor, C-X-C motif chemokine ligand (CXCL) 8, CXCL1 and C-C motif chemokine ligand (CCL) 2, was investigated using western blotting. Kidney renal papillary cell carcinoma mRNA-sequencing data obtained from The Cancer Genome Atlas revealed that chemokines, including CXCL1/2/3, CXCL8, MMP7 and CCL20, were positively correlated with B7-H4 gene expression. Furthermore, 59 clinical renal cell carcinoma tissues were collected and analyzed by immunohistochemical staining. The results revealed the positive correlation of B7-H4 with CCL20 and CXCL8, and validated the DEGs identified in tumor cell lines. 786-O transfectants were inoculated into non-obese diabetic/severe combined immunodeficiency mice, and tumor growth was investigated. B7-H4 overexpression promoted tumor growth and administration of anti-CXCL8 antibody reversed this effect. Furthermore, B7-H4 overexpression increased the number of tumor-infiltrating neutrophils while inhibition of CXCL8 abrogated this effect. These data indicated that recruitment of neutrophils in the tumor microenvironment by CXCL8 serves an important role in the tumor promotion effect of B7-H4. The present study revealed a novel mechanism of B7-H4 in tumor promotion in addition to T cell inhibition.

Introduction

Renal cell carcinoma (RCC) is one of the most common cancers, accounting for ≈140,000 deaths worldwide each year (1). RCC has been considered to be an immunogenic tumor (2). Interleukin-2, interferon α and immune checkpoint inhibitors have been used clinically in RCC therapy (3-5). Novel immunotherapies, such as the combination of anti-programmed death ligand-1 antibody and anti-cytotoxic T-lymphocyte-associated protein 4 antibody, have demonstrated clinical benefit (6). However, only a proportion of patients with RCC benefit from the novel immunotherapies (7). The immune suppression microenvironment of RCC remains to be investigated.

B7 family member H4 (B7-H4), also known as B7x or B7S1, is one of the members of the B7 superfamily of co-stimulatory molecules and serves as an inhibitory modulator of the T-cell response (8). B7-H4 mRNA is widely expressed in human peripheral tissues, but its protein expression in normal tissues seems to be limited (9). To date, B7-H4 has been detected in several types of human cancer tissue. In ovarian cancer, B7-H4 expression is associated with tumor-infiltrated antigen-presenting cells (APCs) (10). In colorectal carcinoma, B7-H4 facilitates tumor proliferation and metastasis (11). The expression of B7-H4 in lung cancer is associated with decreased PFS under nivolumab treatment (12). Elevated
B7-H4 in breast cancer is associated with an ‘immune-cold’ microenvironment (13). In renal cell carcinoma, intrahepatic cholangiocarcinoma and thyroid cancer, the expression level of B7-H4 is positively associated with tumor progression (14-16). Previous studies have reported that B7-H4, which is a type I transmembrane glycoprotein, binds to its corresponding receptor on lymphocytes, thus negatively regulating the immune response (8,17,18). However, certain tumors express B7-H4 protein in intracellular compartments (19-22). Unlike tumor-associated macrophages, B-cells and dendritic cells (DCs) with membrane expression of B7-H4, tumor cells expressing intracellular B7-H4 do not inhibit T-cell immunity (21). Therefore, it appears that intracellular B7-H4 has a distinct biological function to membrane-located B7-H4 (22,23). In order to investigate the biological function of B7-H4 in kidney cancer cells, the present study constructed B7-H4 (22,23). In order to investigate the biological function of B7-H4 in kidney cancer cells, the present study constructed the present study constructed B7-H4 (22,23). In order to investigate the biological function of B7-H4 in kidney cancer cells, the present study constructed B7-H4 (22,23). In order to investigate the biological function of B7-H4 in kidney cancer cells, the present study constructed B7-H4 (22,23).

Materials and methods

Cell culture. The 786-O cell line (catalogue TCHu186) was purchased from the Shanghai Cell Bank of the Shanghai Institute for Biological Sciences. The cells were cultured in RPMI 1640 medium (Gibco, Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, Inc.) at 37°C, 5% CO2. A total of 1x10⁶ 786-O cells/well in 24-well plates were transfected with 50 µl/well LV5-B7-H4 lentivirus (GenePharma Co., Ltd) or empty vector (GenePharma Co., Ltd) with 5 µg/ml polybrene (GenePharma Co., Ltd) according to the manufacturer’s instructions. The stable expression clones were selected in medium containing 0.5 mg/ml G418 (Thermo Fisher Scientific, Inc.) 24 h post-transfection. After 2 weeks, NC/786-O and B7-H4/786-O cells were harvested. Triplicate samples and B7-H4/786-O cells were subjected to functional enrichment analysis.

To knock down B7-H4 expression in breast cancer cell line SK-BR-3, 1x10⁶ cells were seeded in 6-well plates 24 h prior to transfection. When the cells reached 70-80% confluence in each well, they were treated with Opti-MEM Reduced Serum Medium (Gibco, Thermo Fisher Scientific, Inc.) containing 50 nM B7-H4 small interfering (si)RNA (GenePharma Co., Ltd) and 10 µl Lipofectamine™ 2000 (Invitrogen, Thermo Fisher Scientific, Inc.) for 6 h. The cell supernatant was replaced with fresh medium and cells were cultured for 48 h prior to harvesting and subsequent experiments. The B7-H4 siRNA sequences were as follows: Forward, 5’-GCUGGAGCAAUUGCACUCAAUUG(dTdT)-3’ and reverse, 5’-CAAUGAUGAGUGCAAUUGCUCCAGC(dTdT)-3’.

Microarray processing. For total RNA extraction, NC/786-O and B7-H4/786-O cells were harvested. Triplicate samples were prepared for each cell line. Total RNA was extracted using TRIzol reagent (Invitrogen, Thermo Fisher Scientific, Inc.) according to the manufacturer’s protocol. Total RNA was quantified using a NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies; Thermo Fisher Scientific, Inc.) and the RNA integrity was assessed using an Agilent Bioanalyzer 2100 (Agilent Technologies, Inc.). Total RNA was transcribed into double-stranded cDNA and labeled with cyanine-3-cytidine triphosphate. The labeled cDNAs were hybridized onto Agilent Human Gene Expression microarrays (Agilent Technologies, Inc.). The arrays were scanned by the Agilent Scanner G2505C (Agilent Technologies, Inc.). The heatmap of the gene expression in the two cell lines (triplicates of each cell line) was produced by Multixperimental Viewer software version 4.8.1 (MeV development team).

DEG analysis. Feature Extraction software version 10.7.1.1 (Agilent Technologies, Inc.) was used to analyze array images to obtain raw data. GeneSpringGX version 11.0 (Agilent Technologies) was employed for the basic analysis of the raw data, which were normalized with the quantile algorithm. Probes that had ≥10% values in any one of all conditions and had flags in ‘Detected’ were selected for further data analysis. Genes with fold-change ≥2 and P<0.05 calculated by unpaired Student’s t-test between B7-H4/786-O and NC/786-O cells were identified as DEGs.

Functional enrichment analysis. To explore the functions in which DEGs were enriched in the B7-H4/786-O cell line, the identified DEGs were subjected to functional enrichment analysis with the Datasets for Annotation, Visualization and Integrated Discovery version 6.7 (david.abcc.Nicrf.gov).

Western blot analysis. NC/786-O and B7-H4/786-O cells were harvested and lysed in RIPA buffer. RIPA cell lysis buffer (cat. no. P0013B) was purchased from Beyotime Institute of Biotechnology. Total proteins were extracted, concentrations were determined by bichinonic acid assay, separated using 12% SDS-PAGE (100 µg/lane), and the proteins were transferred onto PVDF membranes. Upon blocking in 5% BSA/PBS buffer at room temperature for 30 min, the membranes were blotted with the primary antibodies at 4°C overnight and subsequently washed with PBS-0.05% (v/v) Tween 20 (PBST) three times. The primary antibodies used were as follows: NME1 (1:500; cat. no. 11086-2-AP, Proteintech Group, Inc.); membrane metalloendopeptidase (MME; 1:500; cat. no 18008-1-AP; Proteintech Group, Inc.); vanin 1 (VNN1; 1:300; cat. no. 21745-1-AP; Proteintech Group, Inc.); matrix metalloproteinase 7 (MMP7; 1:300; cat. no. 10374-2-AP; Proteintech Group, Inc.); tumor necrosis factor α (TNF-α; 1:2,000; cat. no. 60291-1-Ig; Proteintech Group, Inc.); human C-X-C motif chemokine ligand (CXCL) 1/2/3 (1 µg/ml; cat. no. MAB276; R&D Systems, Inc.); CXCL8 (1:500; cat. no. 27095-1-AP; Proteintech Group, Inc.); human C-C motif chemokine ligand 20 (CCL20; 1 µg/ml); cat. no. MAB360; R&D Systems, Inc.); B7-H4 (1:500; cat. no. 12080-1-AP, Proteintech Group, Inc.); β-actin (1:5,000; cat. no. 66009-1-Ig; Proteintech Group, Inc.) and α-tubulin (1:2,000; cat. no. 11224-1-AP, Proteintech Group, Inc.) were used. Then, the membranes were incubated with the secondary antibodies at room temperature for 1 h and subsequently washed three times with PBST. The secondary antibodies horse anti-mouse IgG (H&L)-horseradish peroxi-
dase (HRP; 1:1,000; cat. no. 7076;) and goat anti-rabbit IgG (H&L)-HRP (1:1,000; cat. no. 7074) were purchased from Cell Signaling Technology, Inc. The membranes were immersed in ECL detection reagent and imaged with a Gel Doc™ EZ system (Bio-Rad Laboratories, Inc.). β-actin or α-tubulin were used as loading controls. The intensities of the bands were determined by Image Lab software version 4.1 (Bio-Rad Laboratories, Inc.). Three independent experiments were performed.

The Cancer Genome Atlas (TCGA) analysis. RNA-sequencing TCGA data were analysed using the Gene Expression Profiling Interactive Analysis (GEPIA) website (gepia.cancer-pku.cn/index.html), which is an interactive web server for analysing RNA-sequencing expression data from TCGA and Genotype-Tissue Expression databases (24). The correlation between B7-H4 and the expression of chemokine genes CXCL1, CXCL2, CXCL3, CXCL8 and CCL20 was evaluated by the Pearson's correlation test. P<0.05 was considered to indicate a statistically significant difference.

Immunohistochemistry (IHC). The use of clinical samples was approved by the Soochow University Ethical Review Board, and written informed consent was obtained from the patients. A total of 59 specimens fixed with 10% formalin at room temperature for 24 h and embedded in paraffin were collected from the Second Affiliated Hospital of Soochow University were used for IHC analysis. The patients included 36 males and 23 females (mean age, 59 years; age range 39-78 years). All patients had a pathological diagnosis of renal cell carcinoma and underwent radical nephrectomy. Sections (4-µm) were deparaffinized in serial grades of xylene followed by rehydration in sequentially increasing dilutions of ethanol. Upon antigen retrieval was performed by saline sodium citrate (microwave heating for 30 min), endogenous peroxidase was blocked by 3.0% hydrogen peroxide at room temperature for 1 h. Non-specific interactions were blocked using 1.5% blocking serum (Dako, Agilent Technologies, Inc.) in PBS for 10 min at room temperature. Then the sections were incubated with primary antibodies against B7-H4 (1:100; cat. no. 12080-1-AP; Proteintech Group, Inc.); CCL20 (1:200; cat. no. 26572-1-AP; Proteintech Group, Inc.) and CXCL8 (1:200; cat. no. 27095-1-AP; Proteintech Group, Inc.) at 4˚C overnight. The sections were then incubated with a HRP-conjugated goat anti-rabbit secondary antibody (1:50; cat. no. GK600705; Gene Tech Co., Ltd.) at room temperature for 1 h. Then the sections were stained by the avidin-biotin immunoperoxidase method. For negative control staining, the primary antibodies...
**Table I. Upregulated differentially expressed genes involved in the inflammatory response, immune response and cell chemotaxis.**

| Gene symbol | Regulation (BW/NC) | FC (BW/NC) | Regulation (BW/BM) | FC (BW/BM) |
|-------------|--------------------|------------|--------------------|------------|
| IL33        | Up                 | 379.026787 | Up                 | 258.11453  |
| TLR2        | Up                 | 34.398422  | Up                 | 2.3269036  |
| CXCL1       | Up                 | 34.114403  | Up                 | 10.745004  |
| CXCL8       | Up                 | 29.291115  | Up                 | 125.44275  |
| CSF2        | Up                 | 27.370817  | Up                 | 142.67291  |
| CCL20       | Up                 | 17.881693  | Up                 | 26.53977   |
| CCLX2       | Up                 | 15.134712  | Up                 | 29.041739  |
| TNF         | Up                 | 11.655401  | Up                 | 14.347787  |
| NME5        | Up                 | 11.342079  | Up                 | 7.7392936  |
| VNN1        | Up                 | 10.856487  | Up                 | 10.219795  |
| IGF2        | Up                 | 10.503797  | Up                 | 7.646981   |
| MMP7        | Up                 | 10.202435  | Up                 | 3.5069153  |
| CXCL3       | Up                 | 9.860397   | Up                 | 24.532923  |
| PTGS2       | Up                 | 9.696702   | Up                 | 3.1078327  |
| MMP1        | Up                 | 8.226387   | Up                 | 12.556886  |
| CCL2        | Up                 | 5.6037107  | Up                 | 10.624764  |
| CXCL5       | Up                 | 4.761648   | Up                 | 5.3687253  |
| MME         | Up                 | 3.3166819  | Up                 | 5.5750117  |

BW, B7-H4 wild-type/786-O; NC, negative control/786-O; FC, fold-change; BM, B7-H4 nuclear localization sequence mutant/786-O; B7-H4, B7 family member; H4; up, upregulated.

were omitted in the procedure. The sections were observed under a light microscope (CX43; Olympus Corporation) at x400 magnification and evaluated and graded independently by two investigators. The Allred scoring system was used (25).

Statistical analysis. All data are expressed as the mean ± SD from at least triplicate experiments. For the correlation analysis between B7-H4 and upregulated DEGs in TCGA data, the Pearson’s correlation test was used. For the chemokine IHC score, the unpaired Student’s t-test was used to evaluate the difference in two groups (dichotomized according B7-H4 expression in the two cell lines (triplicates of each cell line) is presented in Fig. 1A. In total, 724 upregulated and 804 downregulated DEGs were obtained. The significantly enriched KEGG pathways of upregulated genes in the B7-H4/786-O cell line were analyzed. The KEGG pathways of upregulated genes with P≤0.01 are shown in Fig. 1B. The results revealed that there was a significant association with ‘rheumatoid arthritis’,

**Flow cytometry.** Flow cytometry analysis of tumor infiltrated neutrophils in tumor tissues was performed as described previously (26). Tumor tissues were mechanically dissected into small pieces and further digested with a mixture of Liberase™ TL (Roche Applied Science) and DNase. Cells digested from the tumor tissues were filtered through a 70-µm filter, fixed with 4% paraformaldehyde at room temperature for 15 min, and incubated in PBS containing 1% FBS (cat. no. 10099141; Gibco, Thermo Fisher Scientific, Inc.) and 3 mM EDTA at room temperature for 20 min to block non-specific binding. The cells were incubated with an anti-mouse cluster of differentiation (CD) 16/32 antibody (2.5 µg/10⁶ cells; cat. no. MAB1460; R&D Systems, Inc.) at 4°C for 30 min. Cells were stained with fluorescein isothiocyanate-conjugated CD45 (0.25 µg/10⁶ cells; cat. no. 101207; Biolegend, Inc.) and APC-conjugated lymphocyte antigen 6 complex, locus G antibodies (0.06 µg/10⁶ cells; cat. no. 127613; Biolegend, Inc.) at 4°C for 30 min. Flow cytometry data acquisition was performed on a BD FACSCalibur (BD Biosciences) and analyzed by FlowJo version 7.6 (BD Biosciences).
'legionellosis', 'TNF signaling pathway', 'nucleotide-binding oligomerization domain (NOD)-like receptor signaling pathway', 'NF-κB signaling pathway' and 'chemokine signaling pathway' (Fig. 1B). GO terms enrichment analysis for upregulated DEGs was performed. The top 20 biological processes (BPs) are presented in Fig. 1C. The results revealed that 'inflammatory response', 'immune response', 'cell chemotaxis' and 'cellular response to lipopolysaccharide' were associated with the upregulated DEGs (Fig. 1C).

**Verification of upregulated DEGs by B7-H4 via western blotting.** By GO BP analysis, the predominant BPs of the upregulated DEGs were 'inflammatory response', 'immune response' and 'cell chemotaxis', all of which are associated with the immune characteristics of tumor cells. Further analysis of GO revealed that IL33, TLR2, CXCL1 and CXCL8 were involved in these BPs. The upregulated DEGs involved in these BPs are listed in Table I. Next, the protein level of upregulated DEGs in 786-O transfectants was verified using western blot analysis. Fig. 2A revealed that the protein levels of NME1, MME, VNN1, MMP7, TNF, CXCL8, CXCL1 and CCL20 were upregulated in B7-H4/786-O cells compared with NC/786-O cells. To further confirm the effect of B7-H4 on the expression of these cytokines, the SK-BR-3 cell line was transfected with scramble small interfering (si)RNA and B7-H4 siRNA. Western blot analysis demonstrated that loss of B7-H4 in the SK-BR-3 cell line resulted in decreased MME, MMP7, CXCL8, CXCL1 and CCL20 (Fig. 2B). Both
Figure 3. Association of B7-H4 with the differentially expressed genes is confirmed in clinical renal cell carcinoma tissues and TCGA datasets. (A) RNA-sequencing data of TCGA data analysed using the Gene Expression Profiling Interactive Analysis website. The correlation of B7-H4 gene in kidney renal papillary cell carcinoma was analysed using the Pearson's correlation test. (B) Immunohistochemical analysis of B7-H4, CCL20 and CXCL8 in clinical renal cell carcinoma tissues. Representative images are shown. Magnification x400. Scale bar, 400 µm. (C) Statistical analysis of the CCL20 and CXCL8 levels in B7-H4high and B7-H4low tissues using the Student's t-test. **P<0.01. B7-H4, B7 family member, H4; TCGA, The Cancer Genome Atlas; CCL20, C-C motif chemokine ligand 20; CXCL8, C-X-C motif chemokine ligand.
Table II. Patient characteristics.

| Clinical variable | B7-H4high (n=25) | B7-H4low (n=34) | P-valuea |
|-------------------|-------------------|-----------------|---------|
| Age, years (mean ± SD) | 57.6±9.8 | 60.3±8.3 | 0.267 |
| <60 | 14 | 14 | 0.260 |
| ≥60 | 11 | 20 | |
| Sex | | | 0.891 |
| Male | 15 | 21 | |
| Female | 10 | 13 | |
| Grade | | | 0.010 |
| G1-2 | 16 | 31 | |
| G3-4 | 9 | 3 | |
| T stage | | | 0.200 |
| T1-2 | 17 | 28 | |
| T3-4 | 8 | 6 | |

aStatistical analysis using the Pearson chi-square test. B7-H4, B7 family member, H4; T, tumor.

overexpression and knocking down of B7-H4 in cancer cell lines confirmed that B7-H4 expression led to upregulation of the cytokines that were identified by microarray analysis.

**Discussion**

B7-H4 is an important negative co-stimulatory molecule of the B7 family (8,9,17). B7-H4 protein expression is restricted to activated T cells, B cells, DCs and macrophages (8). Additionally, it has been reported that B7-H4 is upregulated in several types of cancer (28-31), which suggests that B7-H4 has the potential to be used as a biomarker or therapeutic target for tumors (19,31-33). However, other studies revealed that B7-H4 promotes cell proliferation (34,35), invasion and metastasis of tumor cells (15,35,36), enhances leukemia-initiating cell differentiation (23), and is correlated with carcinogenesis and chemoresistance (34,37).

There are multiple mechanisms by which B7-H4 can affect tumor cell biology. Wang et al (34) reported that silencing B7-H4 enhances drug-induced apoptosis by inhibiting the phosphatase and tensin homolog (PTEN)/PI3K/AKT signaling pathway, indicating the role of B7-H4 in chemoresistance and suggesting that it may be an attractive therapeutic target in triple-negative breast cancer. Xie et al (15) demonstrated that B7-H4 induced epithelial-mesenchymal transition, and promoted invasion and metastasis of tumor cells by the activation of the ERK1/2 signaling pathway. Furthermore, upregulated B7-H4 expression was associated with downregulated Bax, upregulated Bel-2 and activation of caspase-3 (15). Qian et al (38) analyzed the microRNA (miRNA) expression profile following B7-H4 knockdown in pancreatic cancer cell line L3.6p1 and noticed that the differentially expressed miRNAs induced by B7-H4 siRNA were mainly involved in the mitogen-activated protein kinase and PI3K/AKT signaling pathways. Chen et al (37) demonstrated that B7-H4 expression is positively correlated with IL6 expression and signal transducer and activator of transcription 3 phosphorylation. Xia et al (23) revealed that B7-H4 is one of the highly expressed immune molecules on human acute myeloid leukemia cells, and promotes the differentiation of leukemia-initiating cells through the PTEN/AKT/hypoxia-inducible factor-1α/REST corepressor 2/runt-related transcription factor 1 signaling pathway (23).

The present study constructed B7-H4 wild-type overexpressing cells to investigate the specific DEGs induced by
B7-H4 wild-type in 786-O cells. The results revealed that there were 704 upregulated and 804 downregulated DEGs. The upregulated DEGs were associated with the inflammatory response, immune response and cell chemotaxis. Of the upregulated DEGs obtained by microarray, the upregulation of NME, MME, VNN1, MMP7, TNF, CXCL8, CXCL1 and CCL20 were confirmed by western blot analysis. Since all these molecules are involved in the inflammatory response, immune response and cell chemotaxis, the current study further examined the chemokine expression in clinical renal carcinoma by TCGA dataset analysis and IHC staining in 59 clinical tumor tissues. The results revealed that there was a
positive correlation between B7-H4 and CCL20 or CXCL8. Furthermore, B7-H4 increased tumor-associated infiltrating neutrophils by upregulating CXCL8, indicating another mechanism in the tumor promoting effect of B7-H4. Similar the results obtained in the present study, Azuma et al (39) revealed a correlation between serum B7-H4 and neutrophil in peripheral blood from the patients with clear cell renal cancer. The present study demonstrated that B7-H4 expression increased tumor-infiltrating neutrophils by upregulating CXCL8 and that blocking CXCL8 reversed this increase. The present study revealed the molecular mechanism underlying the B7-H4-associated increase in tumor-infiltrating neutrophils, and suggested that B7-H4 and CXCL8 might serve as therapeutic targets to remodel the tumor microenvironment.

Besides CXCL8, the chemokine CCL20 is also upregulated in B7-H4-transfected cells. CCL20 is an 8-kDa protein involved in the maintenance of immunological homeostasis (40). T cells, natural killer (NK) cells, B cells and immature DCs are recruited to the tumor by the interaction of CCL20 with CCR6 (41-43). As CCL20 recruits both anti-tumor leukocytes and pro-tumor leukocytes (regulatory T cells, myeloid dendritic cells and NK cells), the role of CCL20 in tumor progression is complex (44). Tumor cells, macrophages and neutrophils produce CXCL1 and recruit myeloid-derived suppressor cells, which suppress the activity of CD8+ T effector cells to prevent tumor cell killing by CD8+ T cells (44,45). Thus, upregulated CXCL1 expression by B7-H4 may contribute to tumor progression (44). Of the upregulated DEGs identified in the present study, MMP7 exhibited the largest fold-change difference. MMPs are a family of enzymes responsible for the degradation of a wide spectrum of extracellular matrix and non-matrix proteins (46). During carcinogenesis, MMPs can regulate the microenvironment and contribute to several critical steps in cancer development via their involvement in cell proliferation, differentiation, apoptosis, invasion, migration and immune surveillance (46-48). Thus, upregulated MMP7 expression by B7-H4 may serve an important role in tumor progression.

In conclusion, the results of the present study revealed that in renal cell carcinoma, B7-H4 may upregulate CXCL1, CXCL8, CCL20 and MMP7 and thus recruit tumor-associated neutrophils.

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Availability of data and materials
The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors’ contributions
LZ designed the study wrote the manuscript. AL performed the cellular experiments. NZ performed the experiments on the clinical samples. ZZ performed the bioinformatics and statistical analyses. AL and YC performed the animal experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The present study was approved and guided by the Ethics Committee of Soochow University (Suzhou, China). All patients provided written informed consent.

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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