

GBP5 as a prognostic biomarker associated with immune cell infiltration, migration and invasion in clear cell renal cell carcinoma

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Abstract. Treatment of advanced clear cell renal cell carcinoma (ccRCC) primarily involves targeted therapy and immunotherapy, however a number of patients develop treatment resistance. Identifying preferred biomarkers and immune-related therapeutic targets is clinically important for the effective treatment of metastatic ccRCC. Due to the unmet clinical need for novel therapeutic approaches, it is important to explore potential biomarkers and their roles in tumor progression. Although guanylate-binding protein 5 (GBP5) expression is notably upregulated in several cancers and promotes tumor progression, its role in ccRCC remains largely unknown. In the present study, GBP5 expression levels were compared between tumors and adjacent normal tissue using data from the Gene Expression Omnibus, Gene Expression Profiling Interactive Analysis 2 and Tumor Immune Estimation Resource (TIMER) databases. The relationship between GBP5 expression and clinicopathological features, along with its diagnostic and prognostic values, was evaluated using R software and the Kaplan-Meier plotter. Gene Ontology term analysis and gene set enrichment analysis were performed to explore the biological functions of GBP5. The GeneMANIA

database was used to investigate the protein-protein interaction networks involving GBP5. The Tumor Immune Single-Cell Hub and TIMER databases were used to evaluate the immune infiltration landscape of ccRCC and the association between GBP5 and immune cell markers. *In vitro*, GBP5 levels were manipulated in renal cancer cells through silencing or overexpressing GBP5 using lentivirus transfection experiments. The migratory and invasive capabilities of the cells were evaluated using wound-healing and Transwell assays. The GBP5 levels in ccRCC tissues were found to be higher compared with those in adjacent tissues. GBP5 expression was associated with the tumor-lymph node-metastasis classification and pathological stage. Furthermore, elevated GBP5 level was associated with a poor prognosis and demonstrated promising diagnostic value. Bioinformatics analysis of GBP5 suggested its involvement in multiple biological processes and its notable association with tumor-infiltrating immune cells. Cellular experiments demonstrated that GBP5 regulates the migration and invasion of renal cancer cells. Overall, GBP5 shows potential as a prognostic indicator and a candidate target for immunotherapy in patients with ccRCC.

Introduction

Kidney cancer remains one of the most prevalent and deadliest tumors in urological oncology, with ~155,702 mortalities and 434,419 new cases recorded worldwide in 2022 (1). Clear cell renal cell carcinoma (ccRCC) accounts for >70% of all kidney cancer cases and is the most common subtype (2). ccRCC typically exhibits aggressive growth, frequent metastasis and substantial immune infiltration, with ~30% of patients experiencing recurrence after total nephrectomy (3,4). Conventional radiation therapy and chemotherapy have demonstrated limited efficacy in the treatment of all renal cell carcinoma subtypes (5). Immunotherapy and targeted therapy have shown considerable potential in patients with ccRCC. However, owing to low response rates, immunotherapy may not be suitable for all individuals (6). In addition, patients receiving targeted therapy are prone to resistance, limiting the efficacy of existing treatments and complicating ccRCC control (7).

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The outcomes of patients with ccRCC are primarily affected by drug resistance and metastasis (8). Therefore, to effectively treat metastatic ccRCC, it is clinically important to identify the most preferable biomarkers and immune-related therapeutic targets.

Human guanylate-binding protein 5 (GBP5), which belongs to the guanylate-binding protein (GBP) family, is classified under the dynamin superfamily of large GTPases that are induced by interferons (9,10). It is regarded as a key regulator of immune responses in neoplastic diseases (9). Studies have shown that GBP5 exerts antiviral activity and influences innate immunity and inflammation (11,12). Due to the notable functions of other members of the GBP family in proliferation and invasion, GBP5 may also serve a major role in malignancy (13). Previous studies have supported the key role of GBP5 in cancer progression. In gastric adenocarcinomas, GBP5 expression is markedly upregulated, contributing to cancer development through the Janus kinase 1 (JAK1)-STAT1/GBP5/C-X-C motif chemokine ligand 8 (CXCL8) signaling circuit (14,15). In addition, GBP5 enhances the migration, invasion and proliferation of glioblastoma cells, promoting glioblastoma progression through the proto-oncogene tyrosine-protein kinase Src/ERK1/2/MMP3 pathway (16). Furthermore, in triple-negative breast cancer cells exhibiting high GBP5 expression, GBP5 knockdown notably reduces the number of migrating cells, the activity of the IFN- γ /STAT1 and TNF- α /NF- κ B signaling axes and the expression of programmed death-ligand 1 (PD-L1) (17).

Previous reports have outlined the relevance of GBP5 in tumor immunity. Several studies have indicated that GBP5 expression is associated with the extent of immune cell infiltration in breast cancer (17), melanoma (18), colon cancer (19), ovarian cancer (20), hepatocellular cancer (21) and small-cell lung cancer (22), emphasizing the role of GBP5 in shaping the tumor immune microenvironment (TME). In oral squamous cell carcinoma, GBP5 serves as an immune-related biomarker that induces NF- κ B activation and facilitates radioresistance, PD-L1 upregulation and tumor metastasis (23). In hepatocellular carcinoma and ovarian cancer, GBP5 not only activates the PI3K-AKT signaling pathway but also modulates tumor metabolism and immune evasion through the regulation of DNA methylation and micro-RNA networks (24,25). Furthermore, in cutaneous melanoma and ovarian cancer, GBP5 induces pyroptosis through the JAK2-STAT1-caspase-1 axis, therefore influencing cell death and immune responses. Overall, GBP5 modulates the immune microenvironment which further influences the prognosis of diverse tumor types (20,26). In ccRCC, immune-suppressive cells, such as regulatory T cells and myeloid-derived suppressor cells, induce immune dysfunction, leading to poor therapeutic outcomes (27). However, the precise function of GBP5 is still not fully understood in ccRCC.

The present study employed R software together with a variety of bioinformatics tools to integrate and analyze data, aiming to comprehensively investigate the role of GBP5 in ccRCC. Specifically, its differential expression patterns, associations with clinicopathological features, prognostic and diagnostic significance, protein-protein interaction networks, biological functions, and potential involvement in

tumor immune cell infiltration were examined. Furthermore, in vitro cellular experiments were performed to validate and further explore the impact of GBP5 on the biological behavior of ccRCC. To the best of our knowledge, the results of the present study demonstrate the biological function and clinical significance of GBP5 and its influence on tumor immunity in ccRCC for the first time. Fig. 1 illustrates the study design and workflow, highlighting the step-by-step process from data acquisition to experimental validation. The present study provides novel insights into the function of GBP5 in ccRCC, potentially offering new biomarkers and therapeutic targets for the diagnosis and treatment of ccRCC.

Materials and methods

Gene Expression Profiling Interactive Analysis 2 (GEPIA2). GEPIA2 (<http://gepia2.cancer-pku.cn/>) analyzes gene expression differences based on tumor samples and normal samples from both The Cancer Genome Atlas (TCGA; <https://portal.gdc.cancer.gov>) and the Genotype-Tissue Expression (<https://www.gtexportal.org>) databases (28). In the present study, this tool facilitated the comparison of GBP5 expression between renal tumor tissue and adjacent normal tissue.

Gene Expression Omnibus (GEO) dataset selection. From the GEO database (<http://www.ncbi.nlm.nih.gov/geo>), two datasets were acquired. GSE53757 (29) included GBP1-6 mRNA data from 72 ccRCC and 72 normal tissues. GSE36895 (30) provided GBP1-6 mRNA data from 23 ccRCC samples and 23 normal tissues. Analysis of GBP1-6 mRNA levels was conducted using these two datasets.

Kaplan-Meier (KM) plotter. As an online tool, the KM plotter (<https://kmplot.com/analysis>) provided survival analysis capabilities for 21 tumor types by associating gene expression profiles with clinical prognosis (31). Using this resource, the KIRC dataset was selected in the pan-cancer module, and the 'auto select best cutoff' option was applied for patient grouping to evaluate the overall survival of GBP1-7.

GeneMANIA database analysis. GeneMANIA (<http://www.genemania.org>) is a publicly accessible tool for analyzing genetic and protein interactions, co-expression, pathways and gene co-localization (32). The potential interactions of GBP5 with related genes were assessed using this platform.

TIMER database analysis. TIMER (<https://cistrome.shinyapps.io/timer/>) is based on six algorithms used to evaluate immune infiltration levels (33). The impact of GBP5 expression on immune cell infiltration in kidney renal clear cell carcinoma (KIRC) was analyzed in the present study. TIMER2.0 (<http://timer.comp-genomics.org/>) was also employed to investigate GBP5 expression differences between tumor tissue and adjacent tissue, alongside its association with immune cell gene markers in KIRC (34).

Tumor Immune Single-cell Hub 2 (TISCH2) database analysis. Based on single-cell RNA sequencing, the TISCH2 database (<http://tisch.comp-genomics.org/>) is designed to analyze the TME at single-cell resolution (35). The distribution and

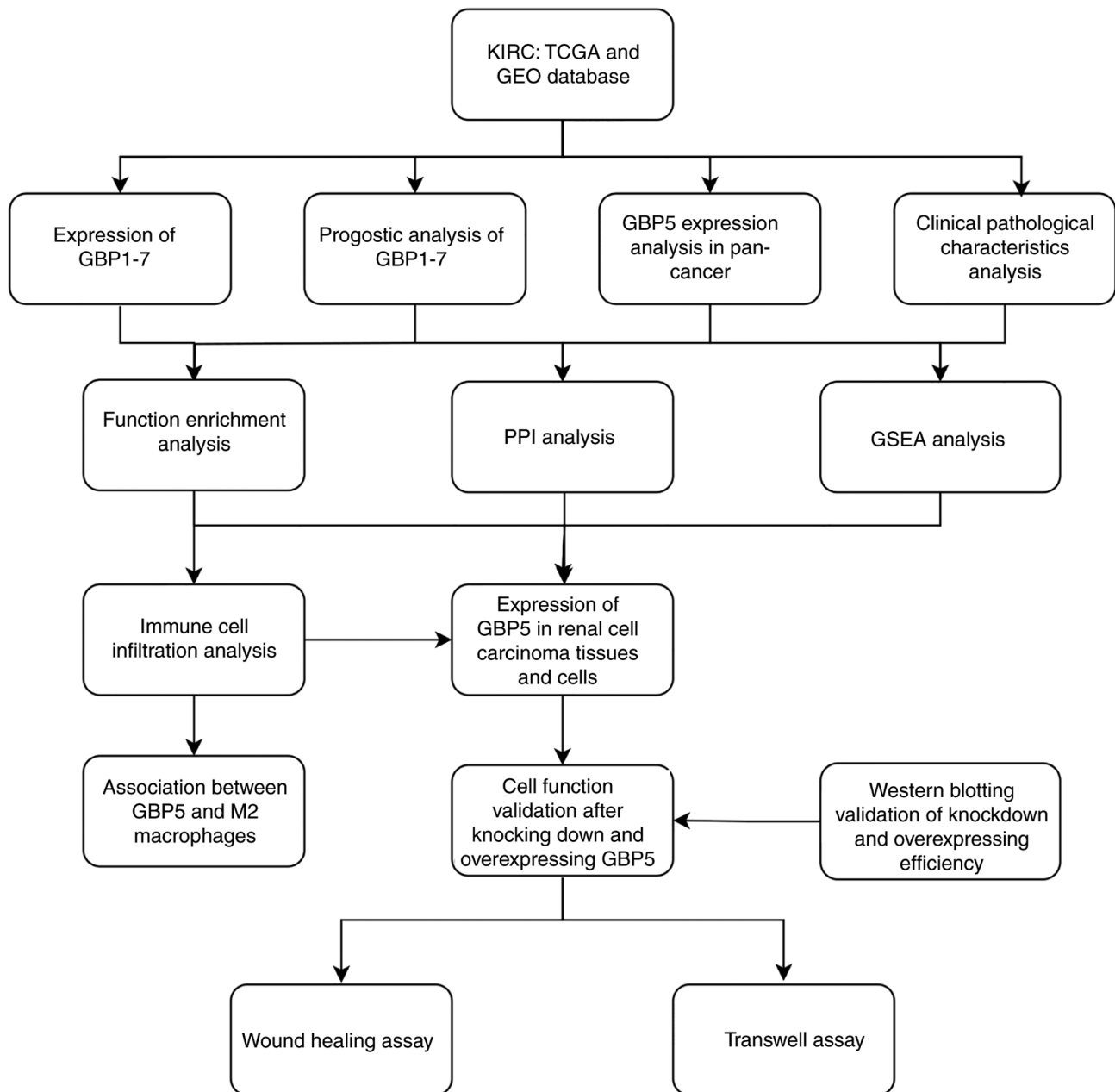


Figure 1. Overall workflow of the study. Schematic representation of the major steps and analytical methods employed, offering a concise and visual summary of the entire research process. KIRC datasets from TCGA and GEO databases were utilized to perform differential gene expression and prognostic analyses of GBP1-7 genes, with a focus on the clinical pathological characteristics of GBP5 in ccRCC, as well as the gene function enrichment analysis, PPI network analysis, GSEA analysis and immune cell infiltration analysis conducted. Finally, wound-healing and Transwell assays were conducted to evaluate the role of GBP5 in the invasion and migration of ccRCC cells. KIRC, kidney renal clear cell carcinoma; TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus; GBP, guanylate-binding protein; PPI, protein-protein interaction; GSEA, gene set enrichment analysis; ccRCC, clear cell renal cell carcinoma.

expression patterns of GBP5 in the TME were analyzed using the GSE111360 (36) and GSE121636 (37) datasets.

Bioinformatics analysis. TCGA-KIRC RNA-sequencing data were downloaded from TCGA database. Transcript per million format expression data and the corresponding clinical information were extracted. Boxplots illustrating GBP5 expression across different clinicopathological features, including tumor-lymph node-metastasis (TNM) stages, were generated using the 'ggplot2 v3.3.5' R package (RStudio, Inc.). Receiver operating characteristic (ROC) curves were plotted to assess the diagnostic value of GBP5 in ccRCC.

Based on the criteria of $\log_2$ fold change ≥ 2 and the adjusted P-value of <0.05 , GBP5-related differentially expressed genes (DEGs) were identified and visualized through a volcano plot plotted using the aforementioned 'ggplot2' R package. Gene Ontology (GO) biological process enrichment analysis was performed using the DAVID database (<https://david.ncifcrf.gov/tools.jsp>). Gene set enrichment analysis (GSEA) was conducted to explore the relevant molecular signaling pathways. Data from the Tracking Tumor Immunophenotype (TIP) database (<http://biocc.hrbmu.edu.cn/TIP>) were used for immune cell infiltration analysis (38). The relationship between GBP5 expression and immune cell infiltration and

circulation was assessed using Spearman's rank correlation analysis.

Clinical samples. A total of eleven pairs of clinical specimens were obtained from the University of Hong Kong-Shenzhen Hospital (Shenzhen, P.R. China) between August 2024 and November 2024. Patients were eligible if no radiotherapy or chemotherapy had been administered prior to biopsy, the pathological diagnosis confirmed ccRCC and no other malignant tumors were present. The Ethics Committee of the University of Hong Kong-Shenzhen Hospital approved the collection of renal carcinoma tumor tissues and adjacent normal tissues (approval no. 2024256), ensuring that all procedures complied with the ethical guidelines of the Declaration of Helsinki. All the donors signed an informed consent form.

Cell culture. Human ACHN, Caki-1, 786-O and human proximal tubular epithelial (HK-2) cell lines were procured from Procell Life Science & Technology Co., Ltd. RPMI 1640 complete medium (Procell Life Science & Technology Co., Ltd.) was used to culture the 786-O and ACHN cells. For Caki-1 cells, McCoy's 5A complete medium (Procell Life Science & Technology Co., Ltd.) was used. For HK-2 cells, Dulbecco's modified eagle medium (Gibco; Thermo Fisher Scientific, Inc.) was utilized. A 10% fetal bovine serum solution (FBS; Procell Life Science & Technology Co., Ltd.) and 1% antibiotics solution (100 µg/ml streptomycin and 100 U/ml penicillin) were added to prepare the complete medium. Cultures were placed at 37°C in a 5% CO₂ incubator, *Mycoplasma* contamination was checked in all cell lines and short tandem repeats analysis was used for authentication.

Transfection of lentivirus. Lentivirus overexpression, GBP5 overexpression (GBP5 OE; 47597-2; order of vector elements: Ubi-MCS-3FLAG-SV40-puromycin), vector controls (CON254), GBP5 short hairpin RNA (shRNA; order of vector elements: hU6-MCS-CBh-gcGFP-IRES-puromycin) and negative control shRNAs (shNC) were produced by Shanghai GeneChem Co., Ltd. The target sequences of the shRNAs were as follows: shNC (CON313), 5'-TTCTCCGAACGTCACGT-3'; the GBP5-specific shRNA shGBP5#1 (103584-1), 5'-CTGGAA ATAGATGGGCAACTT-3' and the GBP5-specific shRNA shGBP5#2 (103585-1), 5'-TGCCTCATCGAGAACTTT AAT-3'. Caki-1 and 786-O cells were transfected according to manufacturer's instructions. Cells infected with lentivirus were treated with puromycin (2 µg/ml) for 7 days, followed by evaluation of target gene knockdown and overexpression efficiency using western blot analysis. The stable cell lines obtained after successful selection and maintained within 50 passages were used for subsequent experiments.

Western blotting. Tissue and cellular protein lysates were prepared using radioimmunoprecipitation assay buffer (cat. no. P0013B; Beyotime Institute of Biotechnology) supplemented with protease and phosphatase inhibitors (cat. no. P1048, Beyotime Institute of Biotechnology). Protein concentrations were determined using the bicinchoninic acid method (cat. no. P0012; Beyotime Institute of Biotechnology). The proteins were resolved using 8-10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis,

the proteins (10-50 µg) were then transferred on to nitrocellulose membranes (cat. no. 66485; Pall Life Sciences). The membranes were blocked with 5% skimmed milk for 1 h at 20-25°C, after which the membranes were incubated at 4°C for 8-12 h with primary antibodies against CD163 (1:1,000; cat. no. R381830; Chengdu Zen-BioScience Co., Ltd.), GAPDH (1:5,000; cat. no. D110016-0100; Sangon Biotech Co., Ltd.), β-actin (1:5,000; cat. no. D110022-0100; Sangon Biotech Co., Ltd.), Flag (1:5,000; cat. no. 20543-1-AP; Proteintech Group, Inc.) and GBP5 (1:2,000; cat. no. 13220-1-AP; Proteintech Group, Inc.). The membranes were incubated with secondary antibodies [horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG; 1:5,000; cat. no. 511203; Chengdu Zen-BioScience Co., Ltd.] and HRP-conjugated goat anti-mouse IgG (1:5,000; cat. no. 511103; Chengdu Zen-BioScience Co., Ltd.) at 20-25°C for 2 h. Protein signals were detected using the ChemiDoc™ MP enhanced chemiluminescence system (Bio-Rad Laboratories, Inc.) and quantified using the ImageJ software (version 1.40 g; National Institutes of Health).

Wound healing assay. 786-O and Caki-1 cells were seeded in 12-well plates and cultured until they reached full confluence. A 1,000 µl pipette tip was used to create a linear scratch in the cell monolayer. The wells were rinsed twice with PBS to remove detached cells and the adherent cells were maintained in a medium containing 1% FBS. Images of the wound area were captured at 0, 10, 12, 24 and 36 h using time-lapse imaging under a light microscope (magnification, x4).

Transwell assay. Transwell assay was performed to assess the migratory potential of ccRCC cells. Cell migration was evaluated using uncoated Transwell chambers (cat. no. 14341, 8 µm pore size; LABSELECT®; Lanjiek Technology Co., Ltd.) and cell invasion was assessed using Matrigel-coated inserts (Corning, Inc.) pre-incubated at 37°C for 30 min. A total of 3x10⁴ 786-O and Caki-1 cells were seeded into the upper chambers of 24-well plates containing serum-free medium. Culture medium supplemented with 15% FBS was added to the lower chambers, making up 500 µl as a chemoattractant. After incubation at 37°C for 24 h for the invasion assay and 12 h for the migration assay, the cells that had moved to the lower surface of the membrane were fixed with 4% paraformaldehyde at 20-25°C for 30 min, followed by crystal violet staining at 20-25°C for 10 min. Following staining, the cells were imaged using a light microscope (magnification, x10) and cell counts were performed in three or more randomly selected fields of view.

Statistical analysis. GraphPad Prism (version 7.0; GraphPad; Dotmatics) was used for statistical analyses. Each experiment was performed in triplicate. Continuous variables are presented as mean ± standard deviation. Both unpaired and paired two-tailed Student's t-tests were used for comparisons between groups. For comparisons involving multiple groups, Tukey's post hoc test was applied after one-way ANOVA. Spearman's or Pearson's correlation coefficients were determined, depending on suitability, to perform correlation analyses. P<0.05 was considered to indicate a statistically significant difference. Additionally, the following criteria

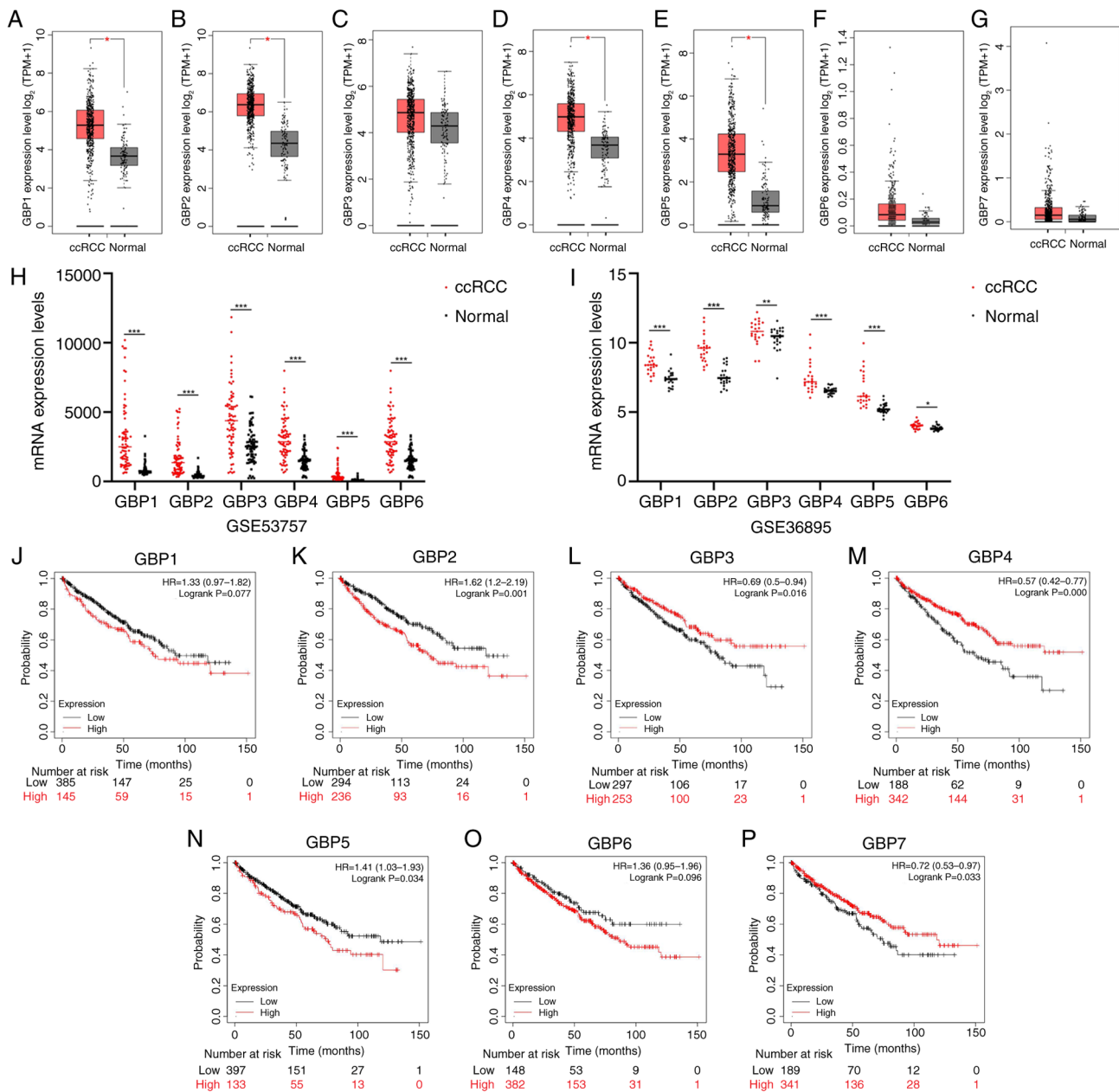


Figure 2. Expression and prognostic analysis of GBP1-7 in ccRCC. (A-G) The expression levels of GBP1-7 in KIRC and matching adjacent tissues were assessed using the gene expression profiling interactive analysis 2 online tool. Results indicate that GBP1, GBP2, GBP4 and GBP5 are highly expressed in KIRC tissues. The mRNA expression of GBP1-6 in ccRCC and adjacent tissues from Gene Expression Omnibus databases (H) GSE53757 (n=72) and (I) GSE36895 (n=23) was analyzed. Results show that GBP1-6 are highly expressed in ccRCC tissues. (J-P) Kaplan-Meier survival analysis results indicate that elevated levels of GBP2 and GBP5 are associated with a poor prognosis, whereas low expression of GBP3, GBP4 and GBP7 is associated with unfavorable outcomes. *P<0.05, **P<0.01, ***P<0.001. GBP, guanylate-binding protein; ccRCC, clear cell renal cell carcinoma; KIRC, kidney renal clear cell carcinoma; TPM, transcript per million; HR, hazard ratio.

indicated statistical significance: *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001.

Results

Role of GBP expression and its prognostic significance in ccRCC. Research suggests that the GBP gene family members regulate the biological behavior of various cancers, indicating their role in cancer initiation and progression (12). Therefore, the GEPIA2.0 tool was initially applied to assess the expression of the GBP family members in ccRCC. Results revealed that GBP1-7 exhibited a consistent expression pattern,

characterized by upregulation in tumor tissues (Fig. 2A-G). Notably, the upregulation of GBP1, GBP2 (39), GBP4 and GBP5 was statistically significant. This observation was consistent with the results of the analysis of ccRCC datasets from the GEO database, further reinforcing their validity (Fig. 2H and I). Furthermore, the Kaplan-Meier survival analysis results indicated that elevated levels of GBP2 (39) and GBP5 were associated with a poor prognosis, whereas low expression of GBP3, GBP4 and GBP7 was associated with unfavorable outcomes (Fig. 2J-P). Collectively, these findings support the hypothesis that the GBP gene family is critically involved in ccRCC, with individual members

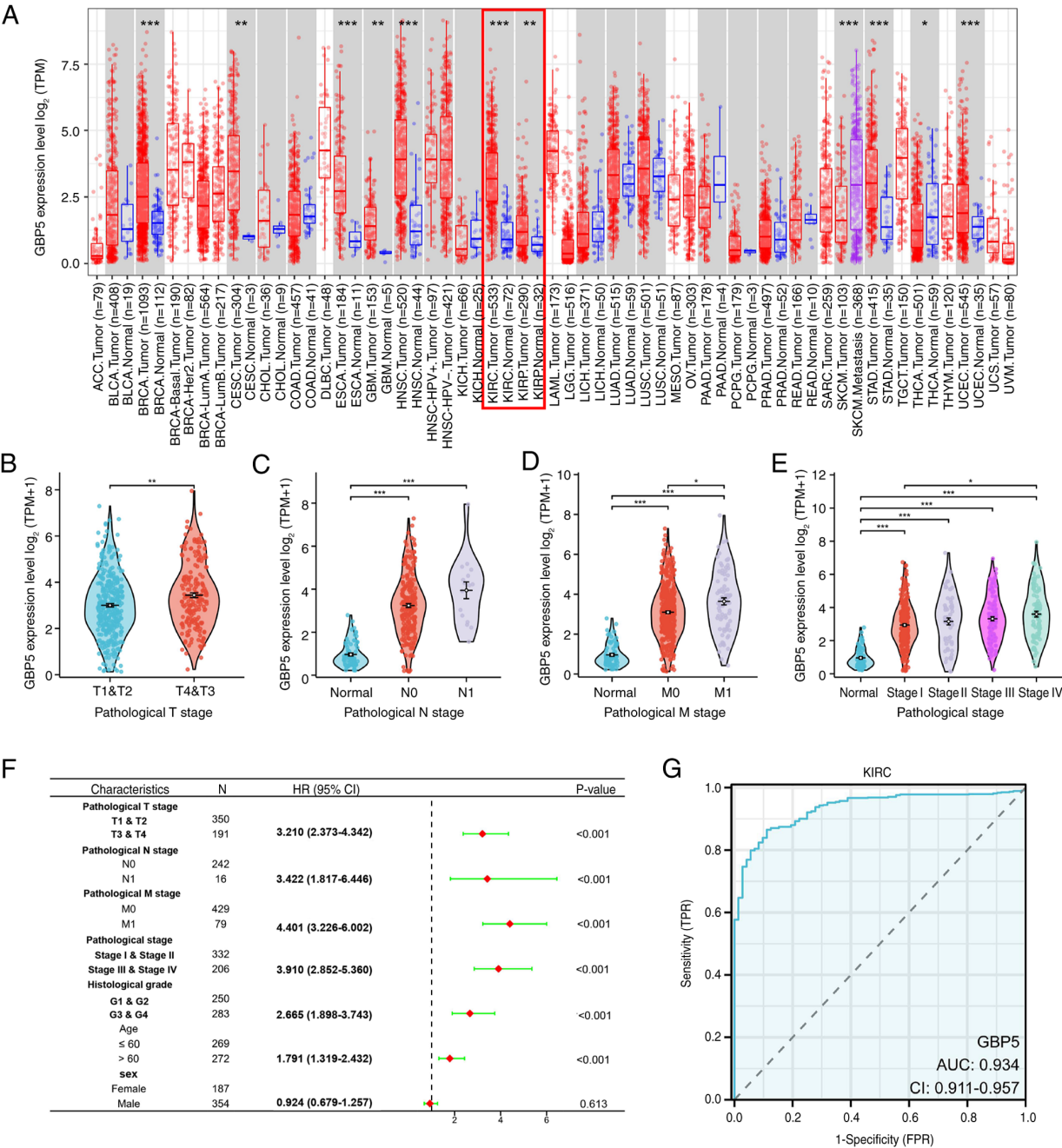


Figure 3. Clinical characteristics of GBP5 in ccRCC. (A) The analysis of GBP5 mRNA expression in tumor and normal tissues was performed using the Tumor Immune Estimation Resource 2.0. Analysis showed a marked increase in GBP5 expression across a wide range of malignancies, notably KIRC and KIRP. The association of GBP5 mRNA expression with different clinical variables in patients with KIRC, such as (B) T stage, (C) N stage, (D) M stage and (E) pathological stage. (F) Univariate cox regression analysis indicates that elevated GBP5 expression is linked to unfavorable clinical characteristics in patients with ccRCC. (G) Receiver operating characteristic curve analysis suggests that GBP5 has high sensitivity and specificity in the diagnosis of ccRCC, with an AUC of 0.934. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$. GBP5, guanylate binding protein 5; ccRCC, clear cell renal cell carcinoma; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; T, tumor; N, lymph node; M, metastasis; AUC, area under curve; TPM, transcript per million; TPR, true-positive rate; FDR, false discovery rate.

exhibiting distinct clinical relevance. Research specifically targeting GBP5 in ccRCC has been limited. Therefore, the present study focused on elucidating the function of GBP5 in ccRCC.

Clinical characteristics and diagnostic value of GBP5 expression in ccRCC. To comprehensively assess the expression profile of GBP5 across multiple tumor types, the TIMER database was employed. Analysis showed a marked

increase in GBP5 expression across a wide range of malignancies, including KIRC and kidney renal papillary cell carcinoma (Fig. 3A). To investigate the clinical relevance of GBP5 in ccRCC, an in-depth analysis was performed using transcriptomic and clinical data from TCGA. Elevated GBP5 expression was significantly associated with notable clinicopathological factors, including tumor size (T stage), lymph node involvement (N stage), distant metastasis (M stage) and both clinical and pathological

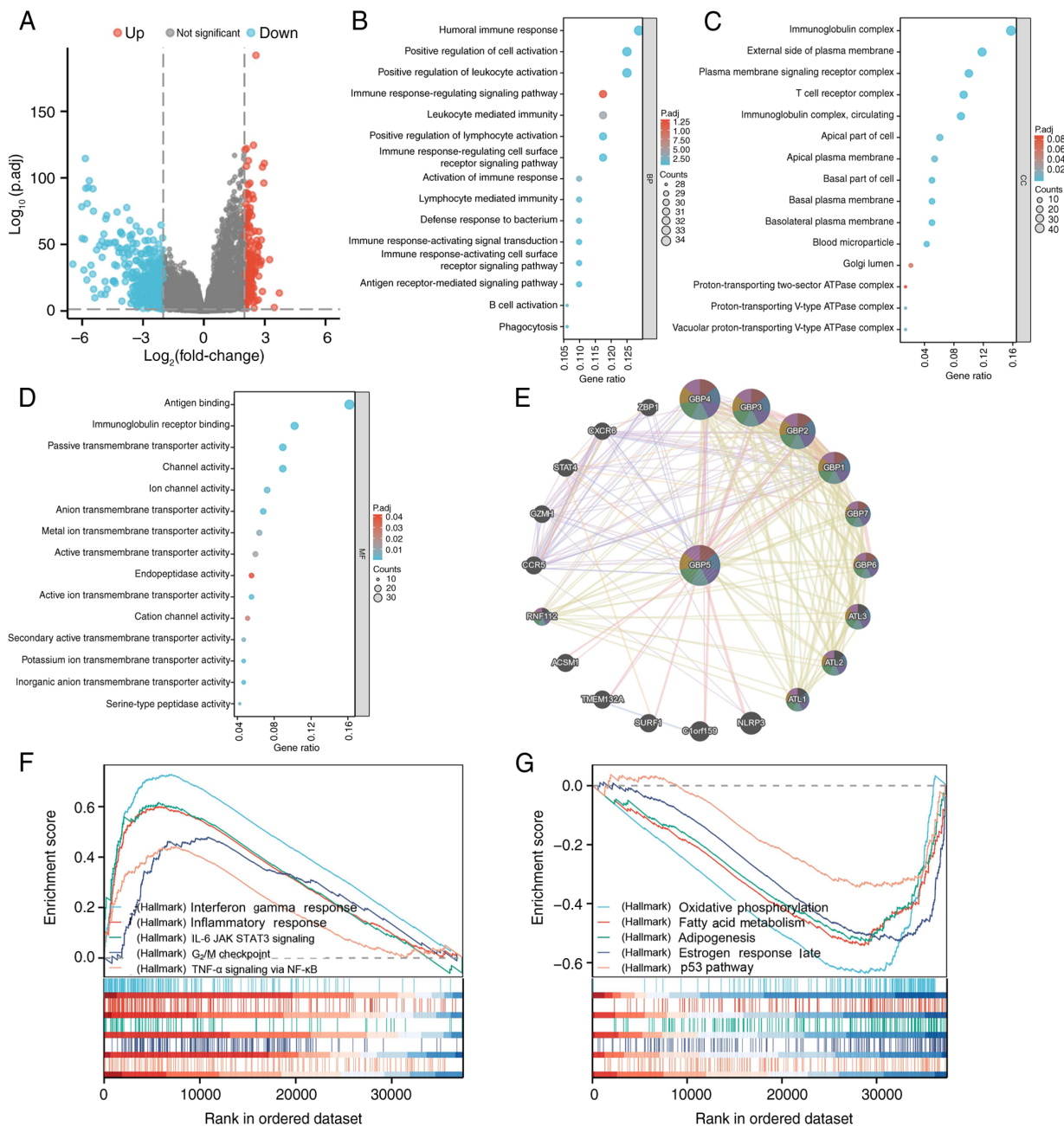
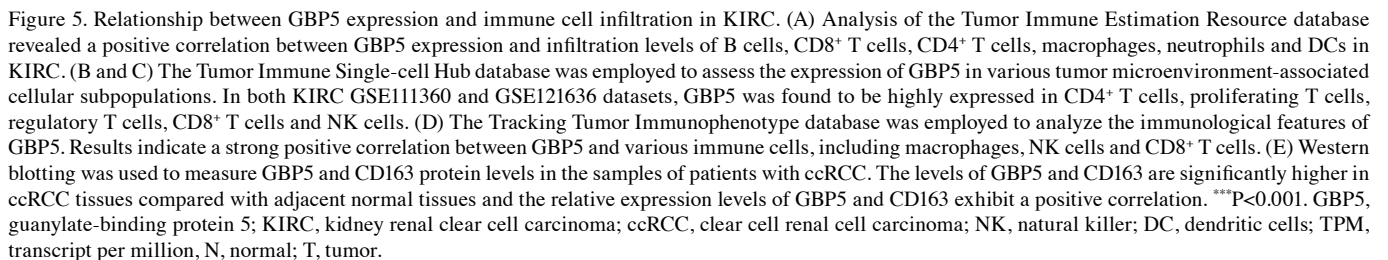


Figure 4. Analysis of functional enrichment of GBP5-related DEGs in KIRC. (A) Volcano plot of DEGs related to GBP5 generated using the Cancer Genome Atlas-KIRC dataset ($\log_2$ fold change >2 ; $P < 0.05$). (B-D) Gene Ontology enrichment analysis of GBP5-related DEGs. (B) Biological process analysis. (C) Cellular component analysis. (D) Molecular function analysis. (E) GeneMANIA protein-protein interaction network for GBP5 illustrates genes as nodes, with the size of each node representing the strength of interaction. The most markedly enriched pathways between the (F) high GBP5 expression and (G) low GBP5 expression groups determined using gene set enrichment analysis. GBP5, guanylate-binding protein 5; DEGs, differentially expressed genes; KIRC, kidney renal clear cell carcinoma; P_{adj}, adjusted P-value.

stage classifications (Fig. 3B-E). Notably, GBP5 expression increased progressively with advancing tumor stage. In addition, univariate Cox regression analysis indicated that elevated GBP5 expression was associated with unfavorable clinical characteristics in patients with ccRCC (Fig. 3F). To evaluate the diagnostic potential of GBP5 in ccRCC, ROC curve analysis was performed, revealing a good diagnostic efficacy with an area under the curve (AUC) value of 0.934 (Fig. 3G), suggesting high sensitivity and specificity. These results imply that GBP5 is a promising diagnostic biomarker involved in the progression of ccRCC.

Functional enrichment of GBP5 interaction and expression related genes. To further investigate the potential biological function of GBP5 in ccRCC, DEG analysis was performed and the results were visualized using a volcano plot (Fig. 4A). Notably upregulated and downregulated genes were identified using the criteria: $\log_2$ fold change >2 and adjusted P-value <0.05 . Enrichment analysis was performed on these DEGs, revealing significant associations with various GO terms. Specifically, GO biological process analysis revealed significant enrichment in 'humoral immune response', 'positive regulation of cell activation',



To further elucidate the biological pathways regulated by GBP5 across the high and low expression groups, GSEA was

Table I. Correlation analysis between guanylate-binding protein 5 and immune cell markers in the Tumor Immune Estimation Resource 2.0 database.

Description	Gene markers	KIRC			
		None		Purity	
		r	P-value	r	P-value
CD8 ⁺ T cell	CD8A	0.838	5.76x10 ⁻¹⁴¹	0.825	6.04x10 ⁻¹¹⁶
	CD8B	0.785	3.79x10 ⁻¹¹⁹	0.767	5.25x10 ⁻⁹⁵
T cell (general)	CD3D	0.837	2.66x10 ⁻¹³⁶	0.819	1.20x10 ⁻¹⁰⁸
	CD3E	0.852	6.79x10 ⁻¹⁵²	0.836	1.73x10 ⁻¹²²
	CD2	0.886	5.95x10 ⁻¹⁷⁵	0.873	2.72x10 ⁻¹⁴²
B cell	CD19	0.474	2.97x10 ⁻³⁴	0.439	4.92x10 ⁻²⁶
	CD79A	0.520	4.93x10 ⁻³⁴	0.486	8.87x10 ⁻²⁶
Monocyte	CD86	0.750	2.52x10 ⁻⁹⁵	0.726	4.68x10 ⁻⁷⁶
	CD115 (CSF1R)	0.604	2.19x10 ⁻⁵⁴	0.556	1.39x10 ⁻³⁹
TAM	CCL2	0.108	1.14x10 ⁻²	0.052	0.263
	CD68	0.470	1.60x10 ⁻¹⁵	0.455	1.05x10 ⁻¹³
	IL10	0.589	3.28x10 ⁻⁵⁰	0.523	2.33x10 ⁻³⁴
M1 Macrophage	INOS(NOS2)	0.046	0.287	-0.02	0.667
	IRF5	0.452	8.40x10 ⁻²⁹	0.444	4.40x10 ⁻²⁴
	COX2(PTGS2)	0.068	0.114	0.028	0.548
M2 Macrophage	CD163	0.512	4.58x10 ⁻³⁹	0.483	0.85x10 ⁻³⁰
	VSIG4	0.501	8.24x10 ⁻³⁷	0.445	1.28x10 ⁻²⁵
	MS4A4A	0.563	4.87x10 ⁻⁴⁶	0.526	5.76x10 ⁻³⁵
Neutrophils	CD66b (CEACAM8)	0.052	0.228	0.056	0.232
	CD11b (ITGAM)	0.576	3.10x10 ⁻⁴⁹	0.529	2.51x10 ⁻³⁵
	CCR7	0.582	1.41x10 ⁻⁴⁸	0.538	5.35x10 ⁻³⁵
Dendritic cell	HLA-DPB1	0.765	6.03x10 ⁻⁹⁹	0.753	1.23x10 ⁻⁸⁰
	HLA-DQB1	0.538	2.18x10 ⁻⁴⁴	0.493	4.34x10 ⁻³⁴
	HLA-DRA	0.778	2.89x10 ⁻¹⁰⁴	0.777	1.45x10 ⁻⁹⁰
	HLA-DPA1	0.773	1.52x10 ⁻⁹⁷	0.768	8.45x10 ⁻⁸³
	BDCA-1 (CD1C)	0.305	4.19x10 ⁻¹³	0.242	9.85x10 ⁻⁸
	BDCA-4 (NRP1)	0.106	2.36x10 ⁻³	0.058	7.69x10 ⁻²
	CD11c (ITGAX)	0.571	3.10x10 ⁻⁴⁷	0.536	2.97x10 ⁻³⁶
Th1	T-bet (TBX21)	0.540	9.41x10 ⁻⁴¹	0.508	6.33x10 ⁻³⁰
	STAT4	0.644	3.19x10 ⁻⁶⁷	0.612	1.20x10 ⁻⁵¹
	STAT1	0.821	3.05x10 ⁻¹²²	0.809	6.13x10 ⁻¹⁰²
	IFN- γ (IFNG)	0.841	3.15x10 ⁻¹⁴⁷	0.823	7.02x10 ⁻¹¹⁷
	TNF- α (TNF)	0.408	1.72x10 ⁻²²	0.368	1.33x10 ⁻¹⁵
Th2	GATA3	0.346	2.95x10 ⁻¹⁶	0.363	1.18x10 ⁻¹⁵
	STAT6	0.142	1.21x10 ⁻⁵	0.152	3.70x10 ⁻⁵
	STAT5A	0.652	6.78x10 ⁻⁶⁴	0.621	1.01x10 ⁻⁴⁹
	IL13	0.112	1.73x10 ⁻²	0.09	0.053
Tfh	BCL6	0.092	0.035	0.089	0.059
	IL21	0.290	4.62x10 ⁻⁴⁴	0.275	1.62x10 ⁻³³
Th17	STAT3	0.268	4.13x10 ⁻¹¹	0.242	3.34x10 ⁻⁸
	IL17A	0.081	0.062	0.063	0.180
Treg	FOXP3	0.678	2.03x10 ⁻⁶⁸	0.651	4.99x10 ⁻⁵⁵
	CCR8	0.734	3.03x10 ⁻⁹⁶	0.719	4.66x10 ⁻⁷⁷
	STAT5B	0.168	2.28x10 ⁻⁵	0.179	4.09x10 ⁻⁵
	TGF β	0.194	2.65x10 ⁻⁶	0.157	3.51x10 ⁻⁴
T cell exhaustion	PDCD1	0.795	3.89x10 ⁻¹¹⁸	0.781	1.82x10 ⁻⁹⁷
	CTLA4	0.773	1.90x10 ⁻¹¹³	0.760	8.78x10 ⁻⁹⁵

Table I. Continued.

Description	Gene markers	KIRC			
		None		Purity	
		r	P-value	r	P-value
	LAG3	0.815	2.78×10^{-119}	0.798	6.42×10^{-99}
	TIM-3 (HAVCR2)	0.371	9.92×10^{-20}	0.331	1.07×10^{-13}
	GZMB	0.489	1.35×10^{-33}	0.444	7.90×10^{-23}

KIRC, kidney renal clear cell carcinoma; None, tumor purity was not used to adjust the results; Purity, in the correlation analysis, the results were corrected by tumor purity; r, correlation coefficient; TAM, tumor-associated macrophages; Th1, T helper type 1 cells; Th2, T helper type 2 cells; Tfh, T follicular helper cells; Th17, T helper 17 cells; Treg, T regulatory cells.

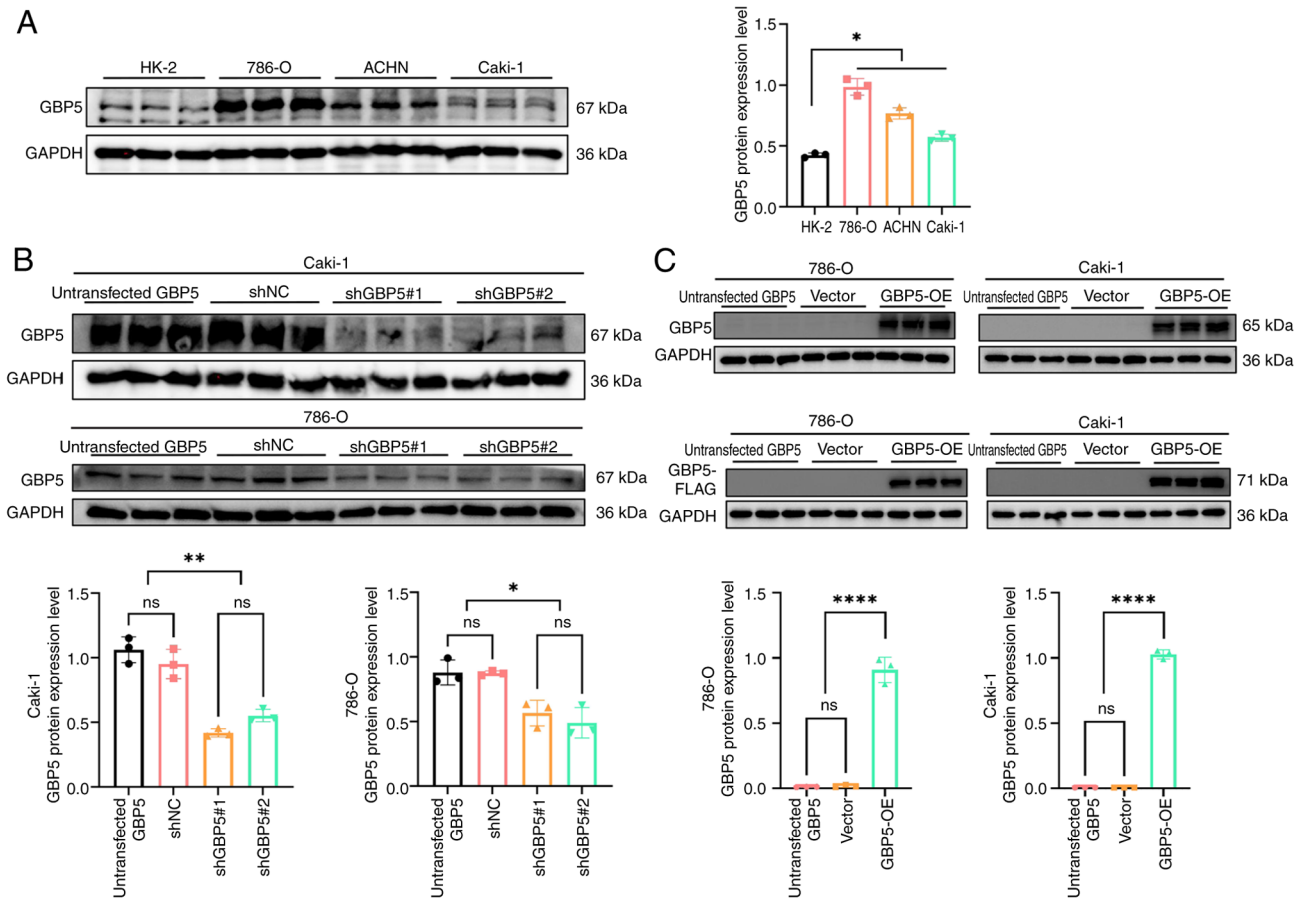


Figure 6. GBP5 levels in renal cell carcinoma cells. (A) The detection of GBP5 expression in HK-2, ACHN, 786-O and Caki-1 cells using western blotting. Results demonstrate that GBP5 protein levels are higher in renal cancer cell lines compared with HK-2 cells. (B and C) The efficiency of knockdown and overexpression of GBP5 in Caki-1 and 786-O cells was determined using western blotting. Results are presented as mean $\pm$ standard deviation from three distinct experiments. * $P < 0.05$, ** $P < 0.01$ and **** $P < 0.0001$. GBP5, guanylate-binding protein 5; OE, overexpression; sh, short hairpin; NC, negative control; ns, not significant.

performed. As shown in Fig. 4F, several biological pathways showed notable enrichment in the high GBP5 expression group, including IFN- γ response, IL-6/JAK/STAT3 signaling, inflammatory response, G₂/M damage checkpoint and TNF- α signaling through NF- κ B. By contrast, pathways such as oxidative phosphorylation, fatty acid metabolism, adipogenesis, estrogen response late and the p53 pathway were enriched in

the low GBP5 expression group (Fig. 4G). According to these findings, GBP5 may influence ccRCC progression through mechanisms related to tumor immunity, cell cycle regulation and inflammation.

Immune cell infiltration is associated with GBP5 in ccRCC.
To investigate whether GBP5 affects immune cell infiltration

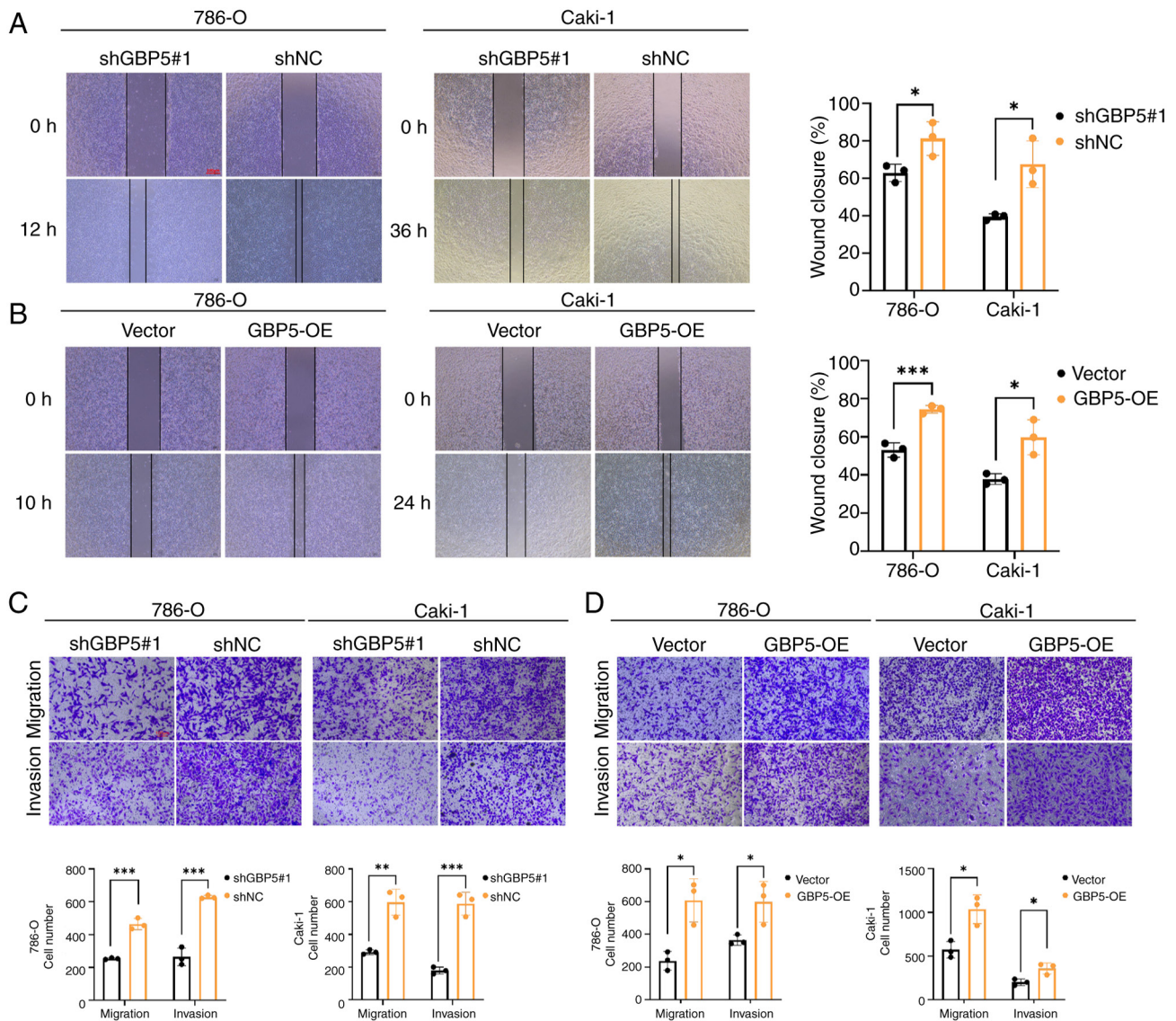


Figure 7. GBP5 enhances clear cell renal cell carcinoma cell migration and invasion. (A and B) The capability of cell migration was assessed through wound healing assay (scale bar, 100 μ m). Results show that the suppression of GBP5 expression significantly decreased the cell migratory capacity and GBP5 overexpression enhanced the wound closure rate. Transwell assays demonstrate that (C) knockdown of GBP5 inhibits cell migration and invasion, whereas (D) GBP5 overexpression significantly promotes migration and invasion (scale bar, 100 μ m). Results are presented as mean $\pm$ standard deviation from three distinct experiments * P <0.05, ** P <0.01 and *** P <0.001. GBP5, guanylate-binding protein 5; OE, overexpression; sh, short hairpin; NC, negative control.

in ccRCC, the TIMER online tool was used to explore the relationship between GBP5 expression and the abundance of various immune cell types. In ccRCC, a strong positive correlation was observed between GBP5 expression and the levels of immune cell infiltration, including B cells, CD8⁺ T cells, CD4⁺ T cells, macrophages, neutrophils and dendritic cells (Fig. 5A). These findings suggest that GBP5 serves a role in immune cell infiltration in ccRCC. TIMER2.0 was then utilized to conduct a deeper analysis of the association between GBP5 expression and marker genes of various immune cells (Table I). The data revealed strong positive correlations between GBP5 and marker genes of T cells, CD8⁺ T cells, M2 macrophages and exhausted T cells, further supporting the involvement of GBP5 in immune regulation in ccRCC.

To validate the relationship between GBP5 expression and immune cell infiltration in ccRCC, two single-cell RNA datasets of ccRCC were analyzed from the TISCH2 database.

GBP5 expression was assessed in different TME-associated cell subsets. The results demonstrated that in both KIRC GSE111360 and GSE121636 datasets, GBP5 was highly expressed in CD4⁺ T cells, proliferating T cells, regulatory T cells, CD8⁺ T cells and natural killer (NK) cells (Fig. 5B and C).

Association between GBP5 and tumor-associated macrophages. Further analysis of the immunological characteristics of GBP5 in ccRCC was conducted. Results indicated a strong positive correlation between GBP5 and various immune cells, including macrophages, NK cells and CD8⁺ T cells (Fig. 5D). To validate the findings from public databases, western blotting of tumor and adjacent normal tissues from 11 patients with ccRCC was performed. In ccRCC tissues, the levels of GBP5 and CD163 were markedly higher compared with those in adjacent normal tissues. The relative expression levels of GBP5 and CD163 exhibited a positive correlation

(Fig. 5E). These findings underscore the association between GBP5 and M2 macrophages and suggest a potential interaction between them.

Verification of GBP5 expression and its effect on the biological behavior of ccRCC. Several *in vitro* experiments were performed to investigate the role of GBP5 in ccRCC cells. Western blotting was performed to assess GBP5 expression in Caki-1, 786-O, ACHN and HK-2 cells. Findings demonstrated that GBP5 protein levels were markedly higher in renal cancer cell lines compared with HK-2 cells (Fig. 6A). For subsequent experiments, 786-O and Caki-1 cell lines were selected.

The efficiency of lentivirus-mediated gene silencing was evaluated using western blotting. As shown in Fig. 6B, two lentiviral constructs carrying different shRNA sequences targeting GBP5 were tested in Caki-1 and 786-O cells. Both shRNA constructs effectively knocked down GBP5 expression, with shGBP5#1 showing improved knockdown efficiency. Thus, the shGBP5#1 lentivirus was selected to establish stable GBP5 knockdown cell lines. Similarly, GBP5 OE cell lines were generated using a lentiviral system. Following the exogenous introduction of GBP5 into 786-O and Caki-1 cells, western blotting analysis confirmed a significant upregulation of GBP5 expression (Fig. 6C).

To explore the biological function of GBP5 in cancer progression, a wound healing assay was performed. Notably, the suppression of GBP5 expression significantly decreased the migratory capacity of the cells (Fig. 7A). By contrast, GBP5 overexpression enhanced the wound-closure rate (Fig. 7B). These findings were consistently observed in Caki-1 and 786-O cell lines, indicating that the GBP5-mediated regulation of cell migration is not cell-type specific.

The Transwell migration assays demonstrated that GBP5 knockdown inhibited cell migration, whereas GBP5 overexpression significantly promoted it (Fig. 7C and D). In addition, the Matrigel-coated Transwell invasion assays demonstrated that GBP5 depletion markedly suppressed the invasive capabilities of Caki-1 and 786-O cells, whereas the overexpression of GBP5 exerted the opposite effect, significantly enhancing cell invasion (Fig. 7C and D).

Discussion

ccRCC is the predominant subtype of RCC. It is associated with a poor prognosis and also lacks effective biomarkers. The primary cause of ccRCC-related mortality is the low rate of early detection and scarcity of effective treatments for patients with advanced or metastatic disease (40,41). Consequently, it is key to identify reliable biomarkers for early detection and prognosis, along with the development of innovative therapeutic approaches to enhance the clinical outcomes of ccRCC.

GBP5, which is induced by IFN- γ and classified within the large GTPase family, is one of seven GBPs with strong sequence homology that contribute to host defense mechanisms (42). The function of GBP5 in cancer, particularly ccRCC, remains largely unclear. In the present study, GBP5 was recognized as a potential immunotherapeutic target, exhibiting upregulation in several types of cancer. Previous studies have highlighted

the involvement of GBP5 in the progression of oral squamous cell carcinoma (23), hepatocellular carcinoma (43), gastric cancer (15) and glioblastoma (16). In the present study, GBP5 was characterized as a novel biomarker for ccRCC involved in the tumorigenesis and progression of this malignancy. Findings show that GBP5 expression was notably increased in ccRCC tissues and was also associated with a poor prognosis. With this, the results of the bioinformatics analysis demonstrated that GBP5 exhibited stage- and grade-specific expression, making it a useful biomarker for diagnosing and predicting the prognosis of patients with ccRCC. However, GBP5 has been reported to serve an opposite role in ovarian cancer (44), likely due to the complexity of ovarian tumorigenesis and the organ-specific characteristics, such as lineage-specific transcription factors (45).

Cancer progression involves a complex array of immune evasion mechanisms that collectively shape the immunological characteristics of the TME. Tumor-associated immune cells have been explored as potential therapeutic biomarkers for cancer (46). In response to pathogen- or danger-associated molecular patterns, GBP5 serves a key role in the activation of the NLRP3 inflammasome (47). It primarily mediates inflammation and activates macrophages in the innate immune system (48). Previous studies have suggested that GBP5 may be involved in tumor immunity (17-22). The efficacy of both targeted therapies and immunotherapies is influenced by the TME immune landscape (49). Various immune cell types such as T cells, regulatory tumor-associated macrophages (TAMs), T regulatory cells (Tregs), cancer-associated fibroblasts and myeloid-derived suppressor cells, serve a role in the TME and contribute to immune cell infiltration, which is regulated by tumor and immune-modulating factors (50,51). Associations between GBP5 expression and several immune cell markers were observed, including CD8⁺ T cells, Tregs, T helper type 1 cells, exhausted T cells, M2 macrophages and monocytes. Patients with ccRCC showing elevated programmed cell death protein 1 expression alongside CD8⁺ T cell and Treg infiltration tended to have worse clinical outcomes (52-54). Tregs may contribute to tumor progression and immune escape by interacting with the TME and suppressing antigen-presenting cell maturation (55). Although CD8⁺ T cell infiltration is elevated in the ccRCC microenvironment, it does not appear to enhance prognosis, most likely because of functional T cell exhaustion (56). Moreover, analysis revealed a positive association between GBP5 expression and CD163, an established marker of M2-polarized TAMs. M2-like TAMs are commonly found in tumors and facilitate tumor progression, cell proliferation, angiogenesis, invasion and metastasis by secreting diverse cytokines and chemokines (57-60). These findings indicate that GBP5 is a possible driver of ccRCC progression through TAMs.

Cancer metastasis is the primary cause of mortality in patients with cancer (61). Regarding cancer biology, GBP5 has been implicated in various malignant behaviors, including tumor invasion, migration, epithelial-mesenchymal transition, cell proliferation and supporting properties of cancer stem cells (16,23). Similarly, GBP5 was found to be involved in the regulation of renal cancer cell invasion and migration, perhaps explaining why patients with high GBP5 expression in ccRCC exhibit a poor prognosis. With regards to the molecular

mechanisms underlying the function of GBP5 in tumorigenesis, previous research has demonstrated that GBP5 might enhance tumor progression in breast and oral cancers by upregulating PD-L1 expression and activating NF- κ B signaling (17,23). Additionally, GBP5 accelerates gastric cancer development by engaging in a JAK1-STAT1/GBP5/CXCL8-mediated positive feedback circuit (15). In the present study, through bioinformatic approaches, GBP5 was found to associate with key oncogenic signaling cascades such as the INF- γ response, IL-6/JAK/STAT3 axis and the TNF- α /NF- κ B network. These pathways are recognized for their essential roles in both cancer development and immune regulation (62,63). However, the precise molecular mechanisms by which GBP5 modulates these pathways in RCC warrants further investigation.

The present study has some limitations. Firstly, the publicly available ccRCC datasets used in the analyses were limited in scope, which may have introduced potential errors or biases. Secondly, while the pro-migratory and pro-invasive roles of GBP5 in renal cancer cells have been confirmed *in vitro*, the underlying molecular mechanisms remain unexplored and no *in vivo* validation has been performed. Finally, although GBP5 appears to be related to tumor immune cell infiltration, the present study lacks supporting *in vitro* and *in vivo* experimental evidence. These limitations must be considered when interpreting the findings and they will be addressed in future studies to provide a deeper understanding of the role of GBP5 in immune cell infiltration and metastasis in ccRCC.

Overall, using bioinformatic analyses and *in vitro* experiments, the upregulation of GBP5 in ccRCC was confirmed. Moreover, immune cell infiltration in ccRCC is associated with the upregulation of GBP5. In addition, GBP5 facilitates renal cancer cell migration and invasion, suggesting its usefulness as a prognostic biomarker for poor clinical outcomes and a promising immunotherapeutic target in ccRCC.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding authors.

Authors' contributions

XC, SJY, XX and CL participated in the study design, conducted the experiments, collected and analyzed the data and drafted the initial manuscript. Bioinformatics analysis and some experimentation was performed by XC, SJY, SY, WZ and ZL. SJY and CL were also in charge of revising

the manuscript, supervising the study and offering technical experimental support. XC, SJY, XX and CL confirm the authenticity of all the raw data. All authors were involved in preparing, proofreading and submitting the manuscript. All authors read, confirmed and approved the final version of the manuscript, and are accountable for the manuscript's content.

Ethics approval and consent to participate

The collection of renal carcinoma tumor tissues and adjacent normal tissues was approved by the Ethics Committee of the University of Hong Kong-Shenzhen Hospital (approval no. 2024256) and was performed in accordance with the guidelines of the Declaration of Helsinki. All donors signed informed consent forms.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I and Jemal A: Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 74: 229-263, 2024.
2. Shuch B, Amin A, Armstrong AJ, Eble JN, Ficarra V, Lopez-Beltran A, Martignoni G, Rini BI and Kutikov A: Understanding pathologic variants of renal cell carcinoma: Distilling therapeutic opportunities from biologic complexity. *Eur Urol* 67: 85-97, 2015.
3. Braun DA, Hou Y, Bakouny Z, Ficial M, Sant' Angelo M, Forman J, Ross-Macdonald P, Berger AC, Jegede OA, Elagina L, *et al*: Interplay of somatic alterations and immune infiltration modulates response to PD-1 blockade in advanced clear cell renal cell carcinoma. *Nat Med* 26: 909-918, 2020.
4. Choueiri TK and Motzer RJ: Systemic therapy for metastatic renal-cell carcinoma. *N Engl J Med* 376: 354-366, 2017.
5. Maines F, Caffo O, Vecchia A, Trentin C, Tortora G, Galligioni E and Bria E: Sequencing new agents after docetaxel in patients with metastatic castration-resistant prostate cancer. *Crit Rev Oncol Hematol* 96: 498-506, 2015.
6. Choueiri TK, Fishman MN, Escudier B, McDermott DF, Drake CG, Kluger H, Stadler WM, Perez-Gracia JL, McNeel DG, Curti B, *et al*: Immunomodulatory activity of nivolumab in metastatic renal cell carcinoma. *Clin Cancer Res* 22: 5461-5471, 2016.
7. Abah MO, Ogenyi DO, Zhilenkova AV, Essogmo FE, Ngaha Tchawe YS, Uchendu IK, Pascal AM, Nikitina NM, Rusanov AS, Sanikovich VD, *et al*: Innovative therapies targeting drug-resistant biomarkers in metastatic clear cell renal cell carcinoma (ccRCC). *Int J Mol Sci* 26: 265, 2024.
8. Linehan WM and Ricketts CJ: Decade in review-kidney cancer: Discoveries, therapies and opportunities. *Nat Rev Urol* 11: 614-616, 2014.
9. Praefcke GJK and McMahon HT: The dynamin superfamily: Universal membrane tubulation and fission molecules? *Nat Rev Mol Cell Biol* 5: 133-147, 2004.
10. Vestal DJ: The guanylate-binding proteins (GBPs): Proinflammatory cytokine-induced members of the dynamin superfamily with unique GTPase activity. *J Interferon Cytokine Res* 25: 435-443, 2005.
11. Shenoy AR, Wellington DA, Kumar P, Kassa H, Booth CJ, Cresswell P and MacMicking JD: GBP5 promotes NLRP3 inflammasome assembly and immunity in mammals. *Science* 336: 481-485, 2012.

12. Tretina K, Park ES, Maminska A and MacMicking JD: Interferon-induced guanylate-binding proteins: Guardians of host defense in health and disease. *J Exp Med* 216: 482-500, 2019.
13. Fellenberg F, Hartmann TB, Dummer R, Usener D, Schadendorf D and Eichmüller S: GBP-5 splicing variants: New guanylate-binding proteins with tumor-associated expression and antigenicity. *J Invest Dermatol* 122: 1510-1517, 2004.
14. Patil PA, Blakely AM, Lombardo KA, Machan JT, Miner TJ, Wang LJ, Marwaha AS and Matoso A: Expression of PD-L1, indoleamine 2,3-dioxygenase and the immune microenvironment in gastric adenocarcinoma. *Histopathology* 73: 124-136, 2018.
15. Cao FY, Wang CH, Li X, Ma MZ, Tao GC, Yang C, Li K, He XB, Tong SL, Zhao QC, *et al*: Guanylate binding protein 5 accelerates gastric cancer progression via the JAK1-STAT1/GBP5/CXCL8 positive feedback loop. *Am J Cancer Res* 13: 1310-1328, 2023.
16. Yu X, Jin J, Zheng Y, Zhu H, Xu H, Ma J, Lan Q, Zhuang Z, Chen CC and Li M: GBP5 drives malignancy of glioblastoma via the Src/ERK1/2/MMP3 pathway. *Cell Death Dis* 12: 203, 2021.
17. Cheng SW, Chen PC, Lin MH, Ger TR, Chiu HW and Lin YF: GBP5 repression suppresses the metastatic potential and PD-L1 expression in triple-negative breast cancer. *Biomedicines* 9: 371, 2021.
18. Deng Z, Liu J, Yu YV and Jin YN: Machine learning-based identification of an immunotherapy-related signature to enhance outcomes and immunotherapy responses in melanoma. *Front Immunol* 15: 1451103, 2024.
19. Elsayed I, Elsayed N, Feng Q, Sheahan K, Moran B and Wang X: Multi-OMICs data analysis identifies molecular features correlating with tumor immunity in colon cancer. *Cancer Biomark* 33: 261-271, 2022.
20. Zou C, Shen J, Xu F, Ye Y, Wu Y and Xu S: Immunoreactive microenvironment modulator GBP5 suppresses ovarian cancer progression by inducing canonical pyroptosis. *J Cancer* 15: 3510-3530, 2024.
21. Xiang S, Li J, Shen J, Zhao Y, Wu X, Li M, Yang X, Kaboli PJ, Du F, Zheng Y, *et al*: Identification of prognostic genes in the tumor microenvironment of hepatocellular carcinoma. *Front Immunol* 12: 653836, 2021.
22. Tong Q, Li D, Yin Y, Cheng L and Ouyang S: GBP5 expression predicted prognosis of immune checkpoint inhibitors in small cell lung cancer and correlated with tumor immune microenvironment. *J Inflamm Res* 16: 4153-4164, 2023.
23. Chiu HW, Lin CH, Lee HH, Lu HW, Lin YHK, Lin YF and Lee HL: Guanylate binding protein 5 triggers NF- κ B activation to foster radioresistance, metastatic progression and PD-L1 expression in oral squamous cell carcinoma. *Clin Immunol* 259: 109892, 2024.
24. Chen HQ, Zhao J, Li Y, He LX, Huang YJ, Shu WQ, Cao J, Liu WB and Liu JY: Gene expression network regulated by DNA methylation and microRNA during microcystin-leucine arginine induced malignant transformation in human hepatocyte L02 cells. *Toxicol Lett* 289: 42-53, 2018.
25. Mitra A, Ghosh S, Porey S and Mal C: GBP5 and ACSS3: Two potential biomarkers of high-grade ovarian cancer identified through downstream analysis of microarray data. *J Biomol Struct Dyn* 41: 4601-4613, 2023.
26. Shi Z, Gu J, Yao Y and Wu Z: Identification of a predictive gene signature related to pyroptosis for the prognosis of cutaneous melanoma. *Medicine (Baltimore)* 101: e30564, 2022.
27. Diaz-Montero CM, Rini BI and Finke JH: The immunology of renal cell carcinoma. *Nat Rev Nephrol* 16: 721-735, 2020.
28. Tang Z, Kang B, Li C, Chen T and Zhang Z: GEPIA2: An enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Res* 47 (W1): W556-W560, 2019.
29. von Roemeling CA, Radisky DC, Marlow LA, Cooper SJ, Grebe SK, Anastasiadis PZ, Tun HW and Copland JA: Neuronal pentraxin 2 supports clear cell renal cell carcinoma by activating the AMPA-selective glutamate receptor-4. *Cancer Res* 74: 4796-4810, 2014.
30. Peña-Llopis S, Vega-Rubín-de-Celis S, Liao A, Leng N, Pavia-Jiménez A, Wang S, Yamasaki T, Zhrebker L, Sivanand S, Spence P, *et al*: BAP1 loss defines a new class of renal cell carcinoma. *Nat Genet* 44: 751-759, 2012.
31. Lánckzy A and Györfy B: Web-based survival analysis tool tailored for medical research (KMplot): Development and implementation. *J Med Internet Res* 23: e27633, 2021.
32. Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, Franz M, Grouios C, Kazi F, Lopes CT, *et al*: The GeneMANIA prediction server: Biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res* 38 (Web Server Issue): W214-W220, 2010.
33. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, Li B and Liu XS: TIMER: A web server for comprehensive analysis of tumor-infiltrating immune cells. *Cancer Res* 77: e108-e110, 2017.
34. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, Li B and Liu XS: TIMER2.0 for analysis of tumor-infiltrating immune cells. *Nucleic Acids Res* 48 (W1): W509-W514, 2020.
35. Han Y, Wang Y, Dong X, Sun D, Liu Z, Yue J, Wang H, Li T and Wang C: TISCH2: Expanded datasets and new tools for single-cell transcriptome analyses of the tumor microenvironment. *Nucleic Acids Res* 51 (D1): D1425-D1431, 2023.
36. Neal JT, Li X, Zhu J, Giangarra V, Grzeskowiak CL, Ju J, Liu IH, Chiou SH, Salahudeen AA, Smith AR, *et al*: Organoid modeling of the tumor immune microenvironment. *Cell* 175: 1972-1988, e16, 2018.
37. Borcherding N, Vishwakarma A, Voigt AP, Bellizzi A, Kaplan J, Nepple K, Salem AK, Jenkins RW, Zakharia Y and Zhang W: Mapping the immune environment in clear cell renal carcinoma by single-cell genomics. *Commun Biol* 4: 122, 2021.
38. Xu L, Deng C, Pang B, Zhang X, Liu W, Liao G, Yuan H, Cheng P, Li F, Long Z, *et al*: TIP: A web server for resolving tumor immunophenotype profiling. *Cancer Res* 78: 6575-6580, 2018.
39. Ye S, Li S, Qin L, Zheng W, Liu B, Li X, Ren Z, Zhao H, Hu X, Ye N and Li G: GBP2 promotes clear cell renal cell carcinoma progression through immune infiltration and regulation of PD-L1 expression via STAT1 signaling. *Oncol Rep* 49: 49, 2023.
40. Cerbone L, Cattrini C, Vallome G, Latocca MM, Boccardo F and Zanardi E: Combination therapy in metastatic renal cell carcinoma: Back to the future? *Semin Oncol* 47: 361-366, 2020.
41. Braun DA, Bakouny Z, Hirsch L, Flippot R, Van Allen EM, Wu CJ and Choueiri TK: Beyond conventional immune-checkpoint inhibition—novel immunotherapies for renal cell carcinoma. *Nat Rev Clin Oncol* 18: 199-214, 2021.
42. Santos JC and Broz P: Sensing of invading pathogens by GBPs: At the crossroads between cell-autonomous and innate immunity. *J Leukoc Biol* 104: 729-735, 2018.
43. Fu J, Qin W, Tong Q, Li Z, Shao Y, Liu Z, Liu C, Wang Z and Xu X: A novel DNA methylation-driver gene signature for long-term survival prediction of hepatitis-positive hepatocellular carcinoma patients. *Cancer Med* 11: 4721-4735, 2022.
44. Zahra A, Dong Q, Hall M, Jeyaneethi J, Silva E, Karteris E and Sisu C: Identification of potential bisphenol A (BPA) exposure biomarkers in ovarian cancer. *J Clin Med* 10: 1979, 2021.
45. Arner EN and Rathmell WK: Mutation and tissue lineage lead to organ-specific cancer. *Nature* 606: 871-872, 2022.
46. Gajewski TF, Schreiber H and Fu YX: Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol* 14: 1014-1022, 2013.
47. Liu P, Ye L, Ren Y, Zhao G, Zhang Y, Lu S, Li Q, Wu C, Bai L, Zhang Z, *et al*: Chemotherapy-induced phlebitis via the GBP5/NLRP3 inflammasome axis and the therapeutic effect of aescin. *Br J Pharmacol* 180: 1132-1147, 2023.
48. Zhou L, Zhao H, Zhao H, Meng X, Zhao Z, Xie H, Li J, Tang Y and Zhang Y: GBP5 exacerbates rosacea-like skin inflammation by skewing macrophage polarization towards M1 phenotype through the NF- κ B signalling pathway. *J Eur Acad Dermatol Venereol* 37: 796-809, 2023.
49. Wilkie KP and Hahnfeldt P: Tumor-immune dynamics regulated in the microenvironment inform the transient nature of immune-induced tumor dormancy. *Cancer Res* 73: 3534-3544, 2013.
50. Heidegger I, Pircher A and Pichler R: Targeting the tumor microenvironment in renal cell cancer biology and therapy. *Front Oncol* 9: 490, 2019.
51. Najjar YG, Rayman P, Jia X, Pavicic PJ Jr, Rini BI, Tannenbaum C, Ko J, Haywood S, Cohen P, Hamilton T, *et al*: Myeloid-derived suppressor cell subset accumulation in renal cell carcinoma parenchyma is associated with intratumoral expression of IL1 β , IL8, CXCL5, and Mip-1 α . *Clin Cancer Res* 23: 2346-2355, 2017.
52. Dai S, Zeng H, Liu Z, Jin K, Jiang W, Wang Z, Lin Z, Xiong Y, Wang J, Chang Y, *et al*: Intratumoral CXCL13⁺CD8⁺T cell infiltration determines poor clinical outcomes and immunoevasive contexture in patients with clear cell renal cell carcinoma. *J Immunother Cancer* 9: e001823, 2021.

53. Giraldo NA, Becht E, Pagès F, Skliris G, Verkarre V, Vano Y, Mejean A, Saint-Aubert N, Lacroix L, Natario I, *et al*: Orchestration and prognostic significance of immune checkpoints in the microenvironment of primary and metastatic renal cell cancer. *Clin Cancer Res* 21: 3031-3040, 2015.
54. Giraldo NA, Becht E, Vano Y, Petitprez F, Lacroix L, Validire P, Sanchez-Salas R, Ingels A, Oudard S, Moatti A, *et al*: Tumor-infiltrating and peripheral blood t-cell immunophenotypes predict early relapse in localized clear cell renal cell carcinoma. *Clin Cancer Res* 23: 4416-4428, 2017.
55. Paluskievicz CM, Cao X, Abdi R, Zheng P, Liu Y and Bromberg JS: T Regulatory cells and priming the suppressive tumor microenvironment. *Front Immunol* 10: 2453, 2019.
56. Braun DA, Street K, Burke KP, Cookmeyer DL, Denize T, Pedersen CB, Gohil SH, Schindler N, Pomerance L, Hirsch L, *et al*: Progressive immune dysfunction with advancing disease stage in renal cell carcinoma. *Cancer Cell* 39: 632-648.e8, 2021.
57. Mantovani A, Marchesi F, Malesci A, Laghi L and Allavena P: Tumour-associated macrophages as treatment targets in oncology. *Nat Rev Clin Oncol* 14: 399-416, 2017.
58. Pittet MJ, Michielin O and Migliorini D: Clinical relevance of tumour-associated macrophages. *Nat Rev Clin Oncol* 19: 402-421, 2022.
59. Xiang X, Wang J, Lu D and Xu X: Targeting tumor-associated macrophages to synergize tumor immunotherapy. *Signal Transduct Target Ther* 6: 75, 2021.
60. Zheng W, Ye S, Liu B, Liu D, Yan R, Guo H, Yu H, Hu X, Zhao H, Zhou K and Li G: Crosstalk between GBP2 and M2 macrophage promotes the ccRCC progression. *Cancer Sci* 115: 3570-3586, 2024.
61. Meirson T, Gil-Henn H and Samson AO: Invasion and metastasis: The elusive hallmark of cancer. *Oncogene* 39: 2024-2026, 2020.
62. Yu H, Pardoll D and Jove R: STATs in cancer inflammation and immunity: A leading role for STAT3. *Nat Rev Cancer* 9: 798-809, 2009.
63. Johnson DE, O'Keefe RA and Grandis JR: Targeting the IL-6/JAK/STAT3 signalling axis in cancer. *Nat Rev Clin Oncol* 15: 234-248, 2018.



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