

Original Article

ITPKA promotes invasive phenotypes in clear cell renal cell carcinoma through functional modulation of PPAR signaling

Ziang Si^{1*}, Jiaxi Zhu^{2*}, Shuaizhi Zhu³, Zengshun Kou⁴, Hai Zhu^{4,5}

¹School of Clinical Medicine, Shandong Second Medical University, Weifang 261000, Shandong, China;

²Department of Biomedical Engineering, Graduate School of Arts and Sciences, Yale University, New Haven, CT 06520, USA; ³Department of Urology, Qingdao West Coast New Area District Hospital, Qingdao 266520, Shandong, China; ⁴Department of Urology, Qingdao Municipal Hospital, Qingdao University, Qingdao 266000, Shandong, China; ⁵University of Health and Rehabilitation Sciences, Qingdao 266000, Shandong, China. *Equal contributors and co-first authors.

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Abstract: Inositol-trisphosphate 3-kinase A (ITPKA) has been implicated in tumor progression; however, its potential role in clear cell renal cell carcinoma (ccRCC) remains insufficiently defined. This study investigated the expression pattern, prognostic relevance, and potential functional associations of ITPKA in ccRCC. Bioinformatic analyses were performed using TCGA and GEPIA2 datasets to evaluate ITPKA expression, clinicopathological relevance, survival associations, immune infiltration patterns, and enriched signaling pathways. Immunohistochemical (IHC) staining was conducted in 20 paired ccRCC and adjacent non-tumor tissue samples to provide exploratory protein-level validation. Functional assays were performed in 786-O cells following ITPKA knockdown or overexpression. Rescue assays targeting PPAR pathway, using the PPAR γ antagonist GW9662 and PPARG overexpression, were used to examine whether PPAR signaling mediates ITPKA-associated cellular phenotypes. ITPKA expression was upregulated in ccRCC tissues and associated with adverse clinicopathological characteristics and unfavorable survival outcomes in the TCGA-KIRC cohort. Exploratory IHC analysis confirmed stronger ITPKA staining in ccRCC tissues than in matched adjacent normal tissues. Immune infiltration analysis suggested a possible association between ITPKA expression and specific immune cell populations, particularly Treg and Th2 cells. In 786-O cells, ITPKA knockdown attenuated malignant cellular phenotypes, including migration and invasion. Rescue assays showed that PPAR γ inhibition with GW9662 partially reversed the effects of ITPKA knockdown, whereas PPARG overexpression attenuated the phenotypic changes induced by ITPKA overexpression. However, the present study does not establish a direct biochemical interaction between ITPKA and PPAR pathway components. In conclusion, ITPKA may serve as a potential biomarker and functional regulator in ccRCC. These findings provide preliminary evidence for an ITPKA-PPAR-related regulatory axis and warrant further validation in larger clinical cohorts and additional experimental models.

Keywords: Clear cell renal cell carcinoma, ITPKA, PPAR signaling, tumor progression, immune infiltration, prognostic biomarker

Introduction

Renal cell carcinoma (RCC) is a highly lethal malignancy of the adult kidney and represents the sixth most frequently diagnosed cancer in men worldwide [1], with the highest incidence observed in Western countries, accounting for about 3% of all cancers [2]. Among the histological subtypes of RCC, clear cell renal cell carcinoma (ccRCC) is the most common, representing approximately 70-75% of all cases [3,

4]. Although many patients with early-stage RCC have achieved favorable outcomes after surgical treatment in the past decade, prognosis remains poor once they enter the advanced stage, with a 5-year survival rate less than 10% on average [5, 6]. As RCC exhibits resistance to conventional radiotherapy, chemotherapy, and hormonal therapy, the identification of novel biomarkers and molecular targets is crucial for early diagnosis, treatment, and prognostic evaluation of ccRCC.

Inositol (1,4,5)-trisphosphate 3-kinase A (ITPKA, or InsP3 kinase) was first described and characterized by Irvine et al. in 1986, and subsequently cloned by Takazawa et al. in 1990 [7, 8]. ITPKA is a cell motility-promoting protein that enhances the metastatic potential of tumor cells [9]. ITPKA is localized on chromosome 15q15, its C-terminal region exhibits high sequence conservation, while the N-terminal region is relatively variable. In addition to regulating InsP4 production, ITPKA modulates cellular plasticity through F-actin binding [10]. Moreover, ITPKA expression is epigenetically upregulated via DNA methylation in several malignancies, including hepatocellular, lung, ovarian, and breast carcinomas, with expression levels correlating positively with disease progression [10-12]. Given the lack of effective therapeutic options for metastatic lung or breast tumors, inhibition of ITPKA activity may provide novel therapeutic opportunities [13]. The mechanisms underlying ITPKA upregulation in tumors are complex and may be regulated by a variety of molecular mechanisms, including DNA methylation, microRNAs, or aberrant transcription factor signaling [14]. Despite its identification as an oncogene, the role of ITPKA in cancer progression remains less well understood compared to other PI3K family members.

Peroxisome proliferator-activated receptors (PPARs) function as nuclear hormone receptors activated by fatty acids and related ligands. Three PPAR isoforms exist in vertebrates (PPAR α , PPAR β , and PPAR γ), exhibiting distinct expression patterns [15]. Each isoform is encoded by a separate gene and binds specific ligands such as fatty acids and prostaglandins. PPAR α regulates genes involved in lipid metabolism in the liver and skeletal muscle, thereby facilitating the clearance of circulating and intracellular lipids and promoting adaptive responses to fasting. PPAR β enhances glucose and lipid metabolism via upregulation of mitochondrial function and fatty acid desaturation; PPAR γ promotes adipocyte differentiation, fatty acid uptake, and lipid droplet storage, thereby increasing systemic insulin sensitivity and reducing ectopic lipid deposition [16-18]. Although PPAR target genes are primarily associated with fatty acid oxidation (FAO) and lipid metabolism, PPARs also play important roles in tumor and immune cells, including metabolic reprogramming, lipid droplet formation, and

macrophage differentiation [4]. Preliminary enrichment analyses indicated that ITPKA-related genes are significantly associated with the PPAR signaling pathway, suggesting that PPAR-related signaling may contribute to ITPKA-associated biological effects in ccRCC, although the directionality and mechanism of this relationship require further functional exploration.

In the present study, we analyzed ITPKA expression and its potential association with various clinicopathological features. ITPKA expression was validated in clinical specimens from 20 patients with ccRCC by immunohistochemistry (IHC), cell scratch assay, Transwell migration and invasion assays, and Western blotting (WB). In addition, a protein-protein interaction (PPI) network was constructed to identify ITPKA-related differentially expressed genes (DEGs). We also evaluated the potential involvement of ITPKA in PPAR pathway and its contribution to ccRCC progression by integrating pathway enrichment and immune infiltration analyses. Overall, this study aimed to explore the potential functional relevance of ITPKA in ccRCC and to provide preliminary evidence for its potential as a biomarker and candidate molecular regulator.

Methods

Data sources and preprocessing

mRNA expression profiles of ITPKA in pan-cancer tissues and corresponding normal tissues were obtained from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases. RNA-seq data from unpaired and paired samples in the TCGA-Kidney Renal Clear Cell Carcinoma (KIRC) cohort were downloaded and processed for subsequent analyses [19]. Kaplan-Meier (KM) survival analysis using clinical information from the TCGA-KIRC cohort with R software (v3.6.3) and the “survival” package. Survival curves were visualized using the ‘survminer’ and “ggplot2” packages. Receiver operating characteristic (ROC) curve analysis was performed using the “pROC” package, and the area under the curve (AUC) was calculated to evaluate the diagnostic performance of ITPKA in ccRCC.

Immune cell infiltration analysis was assessed using the single-sample Gene Set Enrichment

Analysis (ssGSEA) algorithm implemented in the “GSVA” package, based on marker genes representing 24 immune cell populations.

Differential expression analysis of ITPKA

The Wilcoxon rank sum test was used to evaluate differential ITPKA expression across pancreatic tissues. The expression data of ITPKA from paired and unpaired samples were subjected to Shapiro-Wilk normality analysis, followed by Wilcoxon rank sum test. The relationship between ITPKA expression and clinicopathological characteristics in TCGA-KIRC patients were analyzed using the chi-square test. A two-sided p -value <0.05 was considered statistically significant.

Differentially expressed genes (DEGs) were identified using the thresholds of adjusted p -value <0.05 and $|\text{Log}_2\text{-fold change}| > 1$, and the results were visualized using volcano plots. In addition, 100 co-expressed genes were identified with the help of Genemania and STRING databases for PPI network analysis. Cytoscape software was subsequently used to visualize and screen hub-related genes within the PPI network [20].

Functional enrichment analysis

ITPKA was entered into the “General” module of the GEPIA database, and the 100 genes most strongly correlated with ITPKA were filtered out. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using the “clusterProfiler” and “GOplot” packages [21, 22]. Differential expression analysis was performed in the TCGA-KIRC cohort, followed by gene set enrichment analysis (GSEA) [23]. The reference gene set used for GSEA was ‘h.all.v2022.1.Hs.symbols.gmt[Hallmarks]’.

Immune infiltration analysis of ITPKA

The expression matrix for ccRCC patients was obtained from the TCGA database. Immune cell infiltration levels were analyzed using the CIBERSORT algorithm applied to the TCGA-KIRC dataset. Additionally, single-sample Gene Set Enrichment Analysis (ssGSEA) implemented in the “GSVA” package was used to calculate immune cell infiltration scores based on markers for 24 immune cell types [24]. Spearman

correlation analysis was performed to assess the relationship between ITPKA expression and immune cell infiltration. Samples were stratified into two cohorts (high- and low-ITPKA expression) based on median expression, and enrichment scores of different immune cell types were compared between these groups. A significant threshold of $P < 0.001$ was applied for these analyses.

Prognostic analysis and model construction

The Wilcoxon signed-rank sum test was used to evaluate associations between ITPKA expression and clinicopathological features in ccRCC. Kaplan-Meier (K-M) survival analysis was performed using clinical data from TCGA-KIRC with the “survival” package, and the results were visualized using the “survminer” and “ggplot2” packages. ROC analysis was performed using the “pROC” package, and AUC values were calculated to assess the diagnostic performance of ITPKA in ccRCC. AUC values closer to 1 indicate better the diagnostic efficacy. Multifactorial Cox regression analysis was performed to evaluate the effects of ITPKA expression and clinical characteristics on patient survival. Independent prognostic factors identified from multivariate analysis were incorporated into a nomogram to predict 1-, 3-, and 5-year survival probabilities. Calibration analyses and plots were used to assess the predictive accuracy of the nomogram.

Immunohistochemistry (IHC)

Twenty pairs of ccRCC and matched adjacent normal tissues were collected from patients who underwent surgical resection at Qingdao Municipal Hospital. None of the patients had received preoperative anticancer treatment. All specimens were pathologically confirmed and stored at -80°C until use. Tissue samples were fixed in 10% formalin (Sigma-Aldrich) for 24 h, dehydrated through graded ethanol solutions (Sigma-Aldrich), cleared in xylene (Sigma-Aldrich), embedded in paraffin (Sigma-Aldrich), and sectioned at 4–5 μm . After deparaffinization and rehydration, antigen retrieval was performed in 0.01 M sodium citrate buffer (pH 6.0, Sigma-Aldrich) at 95°C for 15–20 min. Endogenous peroxidase activity was blocked using 5% fetal bovine serum (Gibco) for 30 min at room temperature. Sections were then incubated overnight at 4°C with primary antibodies,

followed by incubation with HRP-conjugated secondary antibodies (ZSGB-BIO, Beijing, China) for 30-60 min at room temperature. Immunoreactive signals were developed using a DAB Chromogenic Kit (ZSGB-BIO), and sections were counterstained with hematoxylin (ZSGB-BIO), dehydrated, cleared, and mounted.

Representative images were captured under $\times 200$ or $\times 400$ magnification using a light microscope. Protein expression was evaluated according to the proportion of positively stained tumor cells and staining intensity. The specific scoring method was as follows: 0, no staining; 1, weak staining or moderate-to-strong staining in $\leq 20\%$ of cells; 2, moderate-to-strong staining in 20-40% of cells; and 3, strong staining in $> 40\%$ of cells.

The IHC cohort was designed as an exploratory paired protein-level validation set rather than as an independently powered clinical correlation cohort. Because the primary clinicopathological and survival analyses were performed using the TCGA-KIRC cohort, no formal sample size calculation was conducted for the 20 paired samples included in the IHC analysis. The purpose of this cohort was to determine whether ITPKA protein expression patterns in tumor tissues were consistent with transcriptomic findings. Therefore, associations between ITPKA IHC scores and detailed clinicopathological variables should be interpreted with caution and were not used as the primary basis for prognostic inference.

Cell culture and transfection

The human ccRCC cell line 786-O (Shanghai Jikai Biotechnology Co., Ltd., Shanghai, China) was cultured in RPMI-1640 medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco), 1% nonessential amino acids (Gibco), and 1% penicillin-streptomycin (Gibco) at 37°C in a humidified atmosphere containing 5% CO_2 . Cells were passaged at 80-90% confluence using 0.25% trypsin (Gibco).

For transfection, cells were seeded into 6-well plates and transfected at 50-70% confluence with pLKO.1-Scramble or pLKO.1-shITPKA lentiviral plasmids (GenePharma, Shanghai, China) (shITPKA sequence: 5'-TGGTCAATCTGCCGGGT-

CATAA-3'; shScramble sequence: 5'-GTATAAG-TCAACTGTTGAC-3') using Lipofectamine 2000 (Invitrogen, Thermo Fisher Scientific). Lentiviral supernatant (100 μL) was mixed with transfection reagent, incubated for 15-20 min at room temperature, and added to cultures, followed by medium replacement after 48 h. Stably transfected cells were selected using puromycin (Sigma-Aldrich, St. Louis, MO, USA).

Scratch experiment

Cells were inoculated on six-well plates at a density of 3×10^5 cells/well. After reaching appropriate confluence, a linear wound was generated using a 200- μL pipette tip. Detached cells were removed by rinsing with phosphate-buffered saline (PBS). Photographs were captured at 0, 8, and 24 h using a Motic optical microscope equipped with a digital imaging system. Cell migration ability was evaluated by measuring wound closure at the indicated time points.

Transwell migration and invasion assays

786-O cells in the logarithmic growth phase were harvested at approximately 80% confluence. For migration assays, 2×10^4 to 5×10^4 cells suspended in serum-free medium were seeded into the upper chamber of Transwell inserts, whereas the lower chamber was filled with medium containing 10% FBS as a chemoattractant. For invasion assays, the upper chambers were pre-coated with Matrigel (Corning, Corning, NY, USA) diluted at 1:8 and incubated at 37°C for 30-60 min to allow gel solidification.

Following incubation for 24-48 hours at 37°C in 5% CO_2 , non-migrated or non-invaded cells on the upper membrane surface were removed, and the remaining cells on the lower membrane were fixed with 4% paraformaldehyde and stained with crystal violet for 30 min. After PBS washing, stained cells were counted under a light microscope in five randomly selected fields. The number of migrated or invaded cells were compared between experimental and control groups.

Western blot (WB)

Cells were washed twice with PBS (Gibco) and lysed in RIPA buffer (Beyotime Biotechnology,

Shanghai, China) containing 1 mM phenylmethylsulfonyl fluoride (PMSF, Beyotime) on ice for 10-15 min. Cell lysates were sonicated (20 cycles at 40W for 1s with 2s intervals) and centrifuged at 12,000×g for 15 min at 4°C. The supernatants were collected, and protein concentrations were determined using a BCA Protein Assay Kit (Beyotime). Protein samples were adjusted to a final concentration of 2 µg/µL using lysis buffer. Subsequently, 1/5 volume of 6X loading buffer (Beyotime) was added, and samples were boiled at 100°C for 10 min before storage at -80°C.

For Western blotting, equal amounts of proteins were separated by SDS-PAGE and transferred onto PVDF membranes (Millipore, Billerica, MA, USA) at 200 mA for 120 min at 4°C. Membranes were blocked with TBST (Tris-buffered saline with Tween 20, Beyotime) containing 5% skimmed milk (BD Biosciences, Franklin Lakes, NJ, USA) for 1 hour at room temperature or overnight at 4°C. The membranes were then incubated with primary antibodies against ITPKA (1:1000, ab151462, Abcam, Cambridge, UK), PPARG (1:1000, ab245099, Abcam), PPARG (1:1000, ab209350, Abcam), E-cadherin (1:1000, ab40772, Abcam), N-cadherin (1:1000, ab76011, Abcam), Vimentin (1:1000, ab92547, Abcam), β -actin (1:5000, ab8226, Abcam), and GAPDH (1:5000, ab8245, Abcam) for 2 hours at room temperature or overnight at 4°C. After incubation, membranes were washed four times with TBST for 8 min each. Appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies were subsequently applied, and protein bands were visualized using enhanced chemiluminescence reagents according to the manufacturer's instructions.

PPAR pathway rescue assay

Both pharmacological inhibition and genetic complementation approaches were used to explore whether ITPKA-associated cellular phenotypes were functionally related to PPAR signaling, particularly PPAR γ . Based on GSEA results showing a negative association between ITPKA expression and the PPAR signaling pathway, together with the observation that PPARG showed the strongest correlation among PPAR isoforms, PPAR γ was selected for further rescue experiments. GW9662, a selective and irre-

versible PPAR γ antagonist, was used as the pharmacological tool. The human ccRCC cell line 786-O served as the experimental model. Pharmacological rescue experiment: Stable ITPKA-knockdown (KD) and negative control (NC) 786-O cells were subdivided into three treatment groups: (I) NC + Vehicle (NC cells treated with vehicle only), (II) KD + Vehicle (ITPKA-KD cells treated with vehicle only), and (III) KD + GW9662 (ITPKA-KD cells treated with 10 µM GW9662, a selective, irreversible PPAR γ antagonist). GW9662 (Sigma-Aldrich) was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich) and stored at -20°C. Compounds were administered 24 h after plating, and functional and molecular assays were performed 48 h post-treatment. Genetic Complementation experiment: Wild-type 786-O cells were assigned to three transfection groups: (I) Blank (untransfected control), (II) OE + Vector (co-transfection with ITPKA-overexpression plasmid and empty vector), and (III) OE + PPARG-OE (co-transfection with ITPKA-overexpression plasmid and PPARG-overexpression plasmid). Transfections were conducted using Lipofectamine 2000 when cells reached 50-70% confluence, per manufacturer's instructions. Culture medium was refreshed 48 h post-transfection, and downstream analyses were carried out 24 h later. Cell proliferation, invasion, migration, wound-healing assay, and immunoblotting were conducted according to the protocols described above.

Statistical analysis

Data processing and statistical analysis were performed using R (v3.6.3) and SPSS 24 (IBM, Armonk, NY, USA). Protein bands from Western blot were initially processed by Image Lab software (Bio-Rad, Hercules, CA, USA) and subsequently processed using Photoshop 2024 (Adobe, San Jose, CA, USA) and ImageJ software (NIH, Bethesda, MD, USA).

Data were presented as mean $\pm$ standard deviation (SD) from at least three independent experiments. The Shapiro-Wilk test was used to assess the normality of data distribution. For normally distributed data, comparisons between two independent groups were performed using the Student's t-test, while paired samples (e.g., tumor vs adjacent tissue) were analyzed using the paired Student's t-test. Non-normally

distributed data were compared using the Wilcoxon rank-sum test for two independent groups and the Wilcoxon signed-rank test for paired samples. Comparisons among multiple independent groups were performed using the Kruskal-Wallis test followed by Dunn's post-hoc test. Repeated measures analysis of variance (ANOVA) was used for experiments involving multiple groups with time-dependent measurements (e.g., wound healing assay, CCK-8 proliferation assay). The chi-square test was used to analyze associations between ITPKA expression and categorical clinical data.

Kaplan-Meier method with log-rank test was used for survival analysis. Univariate and multivariate Cox regression analyses were performed to identify independent prognostic factors. Receiver operating characteristic (ROC) curve analysis was used to evaluate diagnostic and prognostic performance. Spearman correlation analysis was used to assess associations between continuous variables.

All statistical tests were two-sided, and $P < 0.05$ indicated statistical significance. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Results

Differential expression of ITPKA in KIRC

Pan-cancer analysis demonstrated that ITPKA was significantly upregulated in 16 malignant tumor types, including ccRCC (KIRC), kidney renal papillary cell carcinoma (KIRP), and prostate cancer (PRAD) ($P < 0.05$, **Figure 1A**). Among the paired pan-cancer datasets, 13 cancer types exhibited significantly higher ITPKA expression in tumor tissues compared with matched adjacent normal tissues, including ccRCC (**Figure 1B**). Analysis of the TCGA-KIRC cohort showed significantly elevated ITPKA expression in ccRCC tissues in both unpaired and paired samples ($P < 0.05$, **Figure 1C, 1D**). Single-gene differential expression analysis screened 2349 DEGs, including 1922 upregulated genes (positive $\log_2FC > 1$) and 427 downregulated genes (negative $\log_2FC < -1$) (**Figure 1E**).

ROC curve analysis demonstrated that ITPKA expression showed a significant discriminatory performance for ccRCC (AUC=0.898, **Figure 2A**), suggesting its potential as a diagnostic

biomarker. To provide preliminary protein-level support of the transcriptomic findings, IHC staining was performed in 20 paired ccRCC and adjacent non-tumor tissue samples. ITPKA staining intensity was stronger in tumor tissues than in matched adjacent tissues, consistent with the expression pattern observed in the TCGA-KIRC transcriptomic analysis (**Table 1** and **Figure 2B**). Given the exploratory nature and limited sample size of IHC cohort, these findings were not used to establish definitive correlations between ITPKA protein expression and clinicopathological features.

Correlation analysis of ITPKA in KIRC

The top 100 related genes co-expressed with ITPKA were identified using the Gene MANIA and STRING databases, and the PPI networks were plotted, respectively (**Figure 3A, 3B**). Using the GEPIA2 database with a threshold of $|\text{Pearson } R| > 0.55$, 100 genes significantly associated with ITPKA were identified and visualized in a co-expression heatmap (**Figure 3C**). Correlations between the DEGs and clinicopathological characteristics, including TNM stage and pathologic grade, are shown in **Figure 3D**.

Enrichment analysis and the search of ITPKA-related pathways

GSEA enrichment analysis of ITPKA-associated DEGs was performed to identify related signaling pathways. The five pathways showing the strongest negative correlation included PPAR signaling pathway, fatty acid metabolism, and peroxisome-related pathways, whereas the pathways showing the strongest positive correlation included P53 signaling pathway, ribosome and maturity-onset diabetes of the young (MODY) pathway (**Figure 4A, 4B**). Subsequent GSEA on PPAR pathway demonstrated significant negative enrichment (NES=-2.165, **Figure 4C**). In addition, GO and KEGG enrichment analyses of ITPKA-related DEGs showed enrichment in biological processes and pathways associated with regulation of transport, synaptic signaling, neuron projection, somatodendritic compartment, metal ion transmembrane transporter activity, oxytocin signaling pathway, and circadian entrainment (**Figure 4D-H**). Further correlation analysis between ITPKA and PPAR signaling pathway revealed that among the three PPAR family members, PPARG exhib-

ITPKA as a biomarker in clear cell renal cell carcinoma

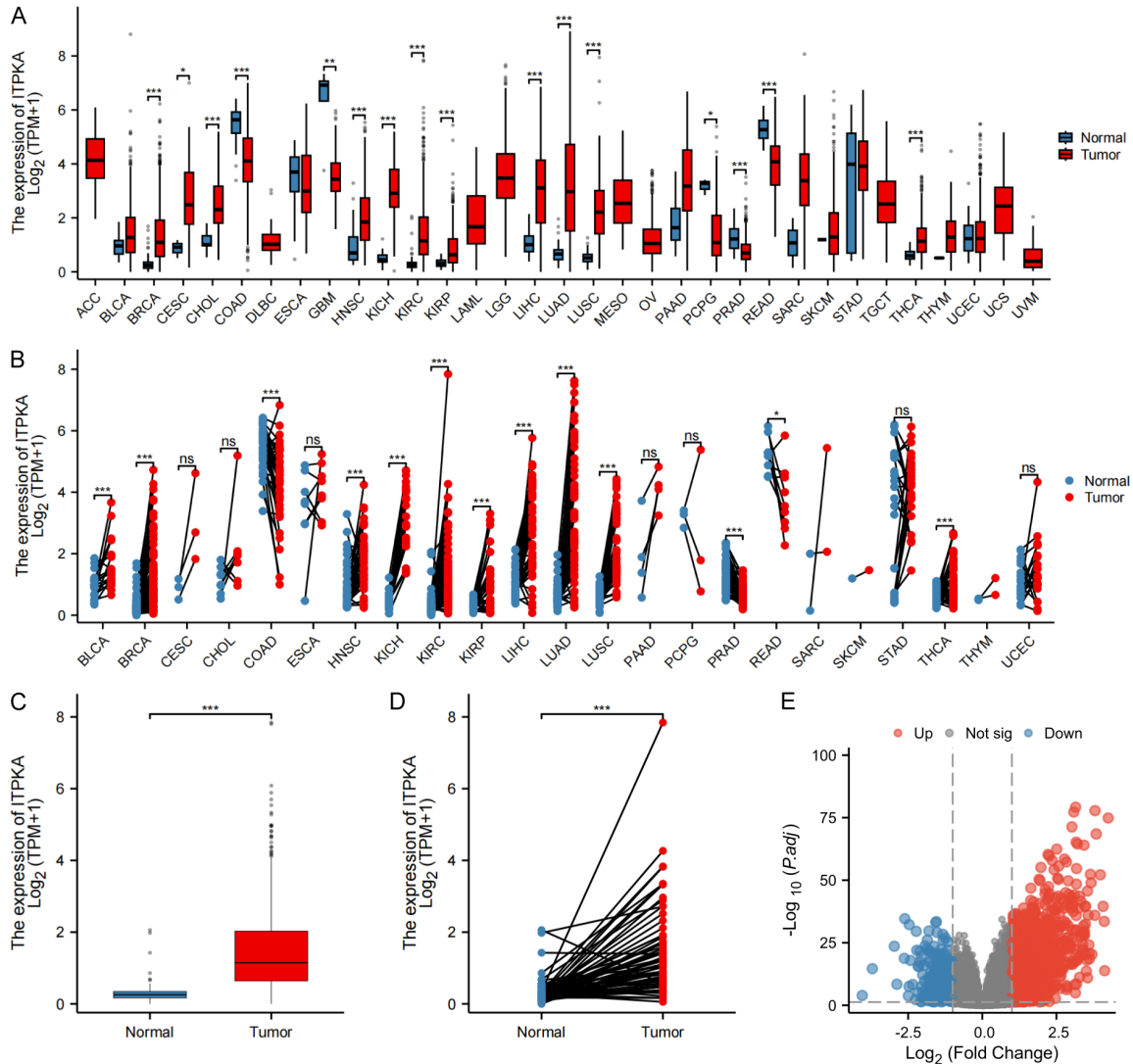


Figure 1. Differential expression of ITPKA in pan-cancer and KIRC cohort. (A, B) ITPKA expression in pan-cancer datasets, showing significant differences between (A) unpaired samples and (B) paired tumor-normal samples. (C, D) ITPKA expression in the TCGA-KIRC cohort, showing significant differences between (C) unpaired tumor and normal samples and (D) paired tumor-normal samples. (E) Single-gene differential expression analysis of ITPKA in KIRC cohort, with blue dots indicating downregulated genes and red dots indicating upregulated genes. Notes: TCGA, The Cancer Genome Atlas. ns; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

ited the strongest correlation with ITPKA expression ($R = -0.457$, **Figure 4I**).

Relationship between ITPKA expression and immune infiltration

Significant differences in immune infiltration scores between high and low ITPKA expression groups were observed in multiple immune cell types, including Th2 cells, activated dendritic cells (aDCs), DCs, macrophages, neutrophils, natural killer (NK) cells, Th1 cells, Th17 cells, and Treg cells (all $P < 0.05$, **Figure 5A**). ITPKA

expression was positively correlated with the estimated enrichment scores of Treg cells, Th2 cells, Th1 cells, macrophages, and several other immune cell subsets, but negatively correlated with Th17 cells and neutrophils (**Figure 5B-D**, $P < 0.05$). A correlation heatmap depicting the association between ITPKA expression and immune cell infiltration scores was generated (**Figure 5E**). Among the immune cell types, ITPKA correlated significantly with tumor-infiltrating Treg cells and Th2 cells ($P < 0.001$, **Figure 5F, 5G**). These results suggest that ITPKA expression is associated with computationally

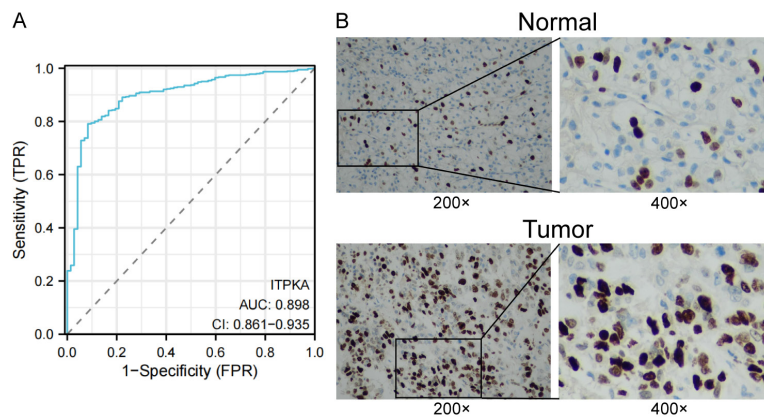


Figure 2. ITPKA protein expression in TCGA-KIRC cohort. A. ROC curve analysis for ITPKA in the TCGA-KIRC cohort; B. Comparison of IHC scores between tumor tissues and matched adjacent normal tissues from 20 patients (Wilcoxon signed-rank test, $P < 0.05$). Notes: ROC, receiver operating characteristic; IHC, immunohistochemistry.

Table 1. Clinical information of 20 patients with clear cell renal cell carcinoma

Patient number	Age	Pathologic stage	Pathologic T stage	IHC
1	70	I	T1	+
2	65	I	T1	+
3	74	I	T1	+
4	62	II	T2	+
5	59	III	T2	+
6	73	II	T2	+
7	82	II	T2	+
8	63	IV	T3	+
9	65	III	T3	++
10	86	III	T3	+
11	77	III	T3	+
12	84	IV	T3	++
13	60	III	T3	++
14	71	IV	T3	+++
15	76	III	T3	++
16	83	IV	T4	+++
17	77	IV	T4	++
18	69	IV	T4	++
19	75	IV	T4	+++
20	81	IV	T4	+++

estimated immune infiltration patterns in ccRCC.

Association of ITPKA expression with clinicopathological characteristics and prognosis in the TCGA-KIRC cohort

Kaplan-Meier analysis showed high ITPKA expression as associated with significantly

worse progression-free interval (PFI), overall survival (OS), and disease-specific survival (DSS) compared with low ITPKA expression (PFI: HR = 3.11, $P < 0.001$, **Figure 6A**; OS: HR = 2.46, $P < 0.001$, **Figure 6B**; DSS: HR = 3.90, $P < 0.001$, **Figure 6C**). Clinical baseline data were obtained from 541 ccRCC (TCGA-KIRC) patients, and ITPKA expression was associated with clinicopathologic features (**Table 2**). Specifically, ITPKA levels were significantly elevated in late-stage disease (III-IV) compared to early-stage disease (I-II) (**Figure 6D**). In TNM staging, ITPKA expression was similarly elevated in T3- T4 stages relative to in T1-T2 stages (**Figure 6E**).

ROC curve analysis based on these clinical data showed moderate predictive performance for ITPKA (AUC=0.699, **Figure 6F**). Multiple prognostic factors were selected for further multivariate Cox regression analysis, based on which a prognostic nomogram incorporating TNM staging, age, and ITPKA level was constructed to estimate survival probabilities in ccRCC patients. The nomogram achieved a concordance index (C-index) of 0.773 (0.750-0.796) (**Figure 6G**). Calibration plots (**Figure 6H**) demonstrated good agreement between predicted and observed outcomes, with the correction line closely aligned to the

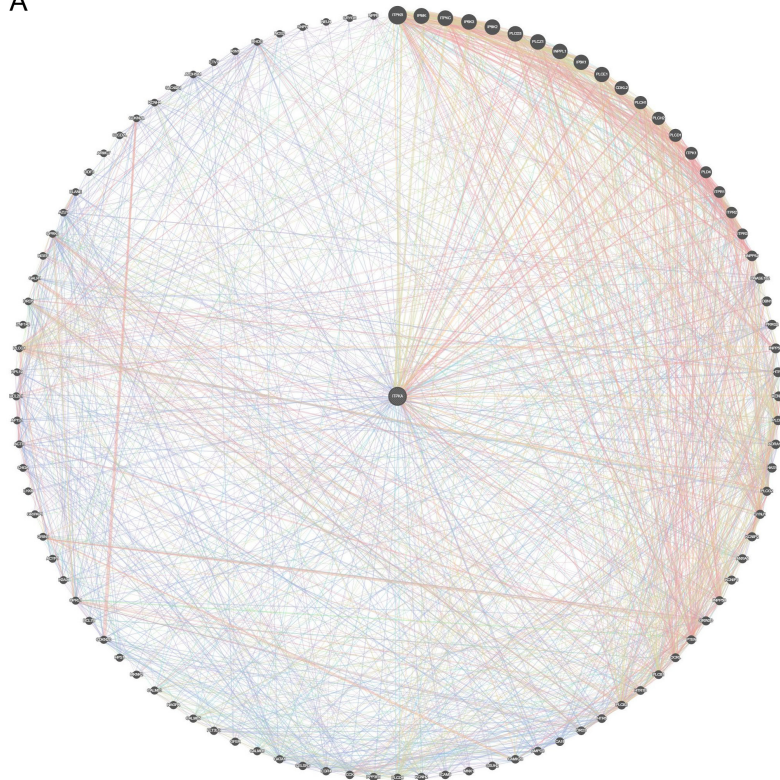
ideal curve (45°), indicating robust predictive accuracy.

ITPKA knockdown suppressed malignant phenotypes of 786-O cells

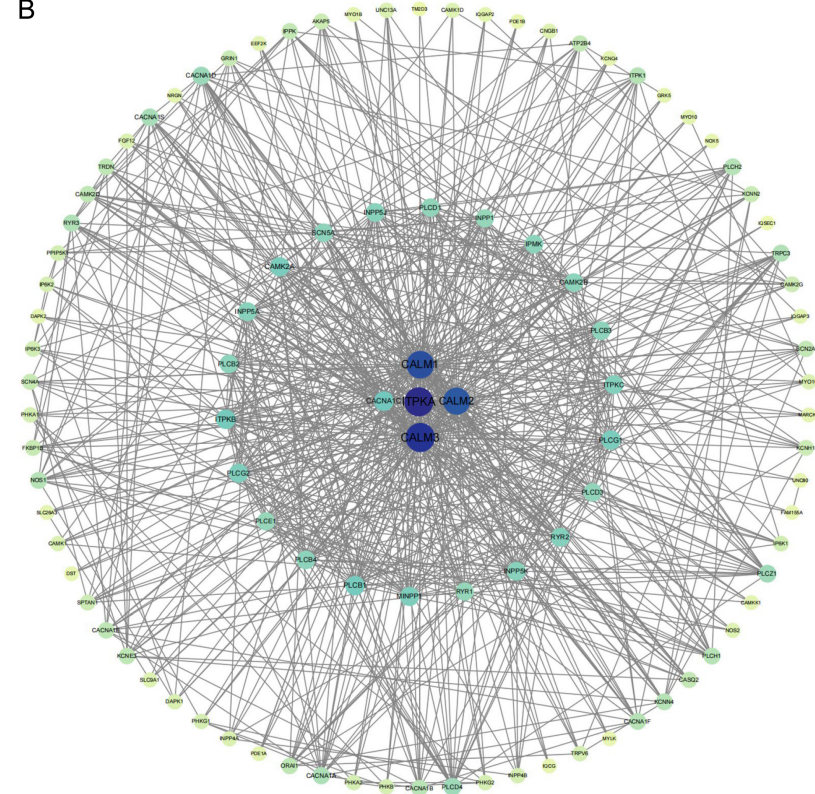
Wound-healing assays demonstrated that ITPKA knockdown markedly impaired wound closure in 786-O cells compared with the nega-

ITPKA as a biomarker in clear cell renal cell carcinoma

A



B



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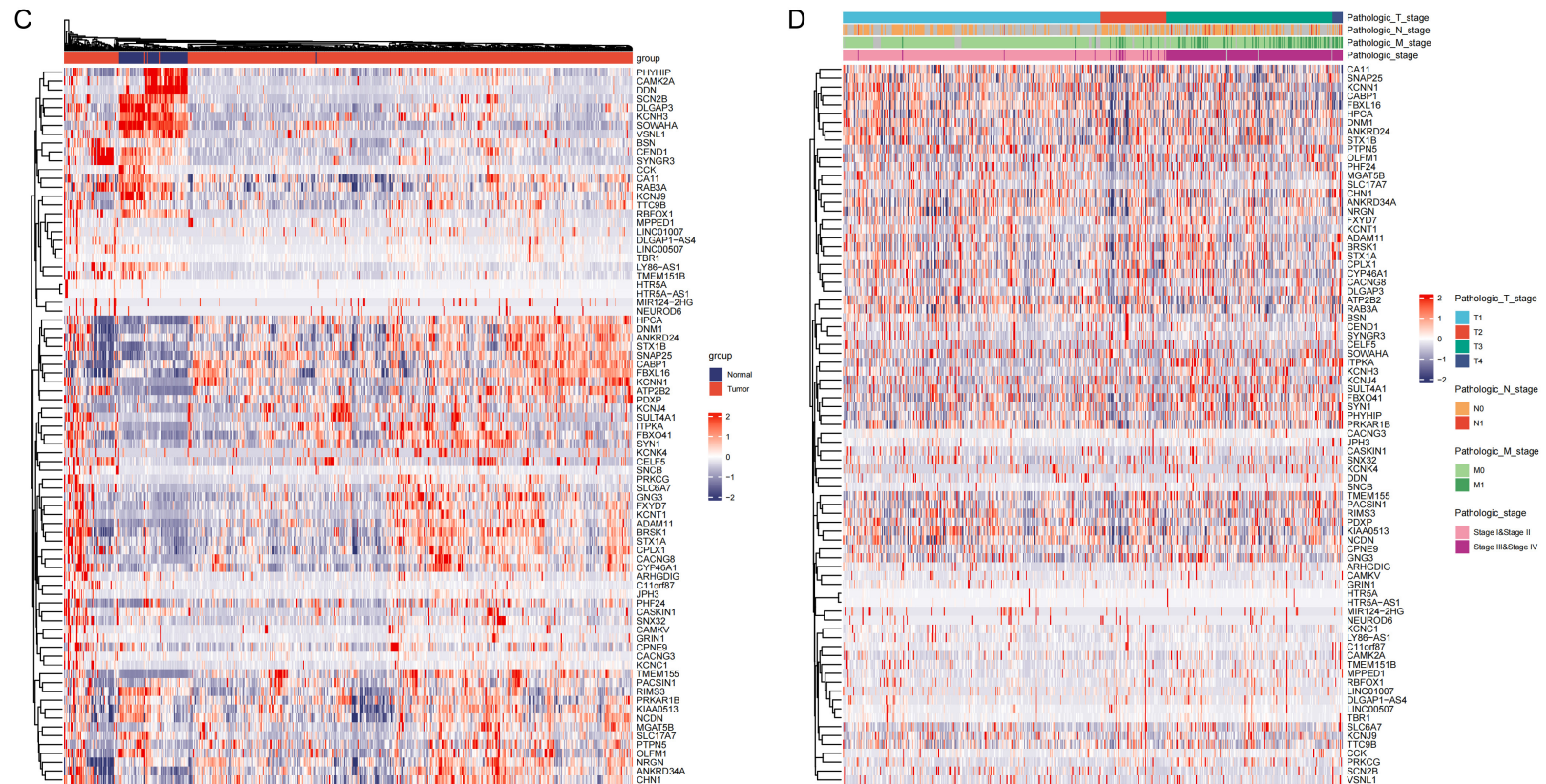
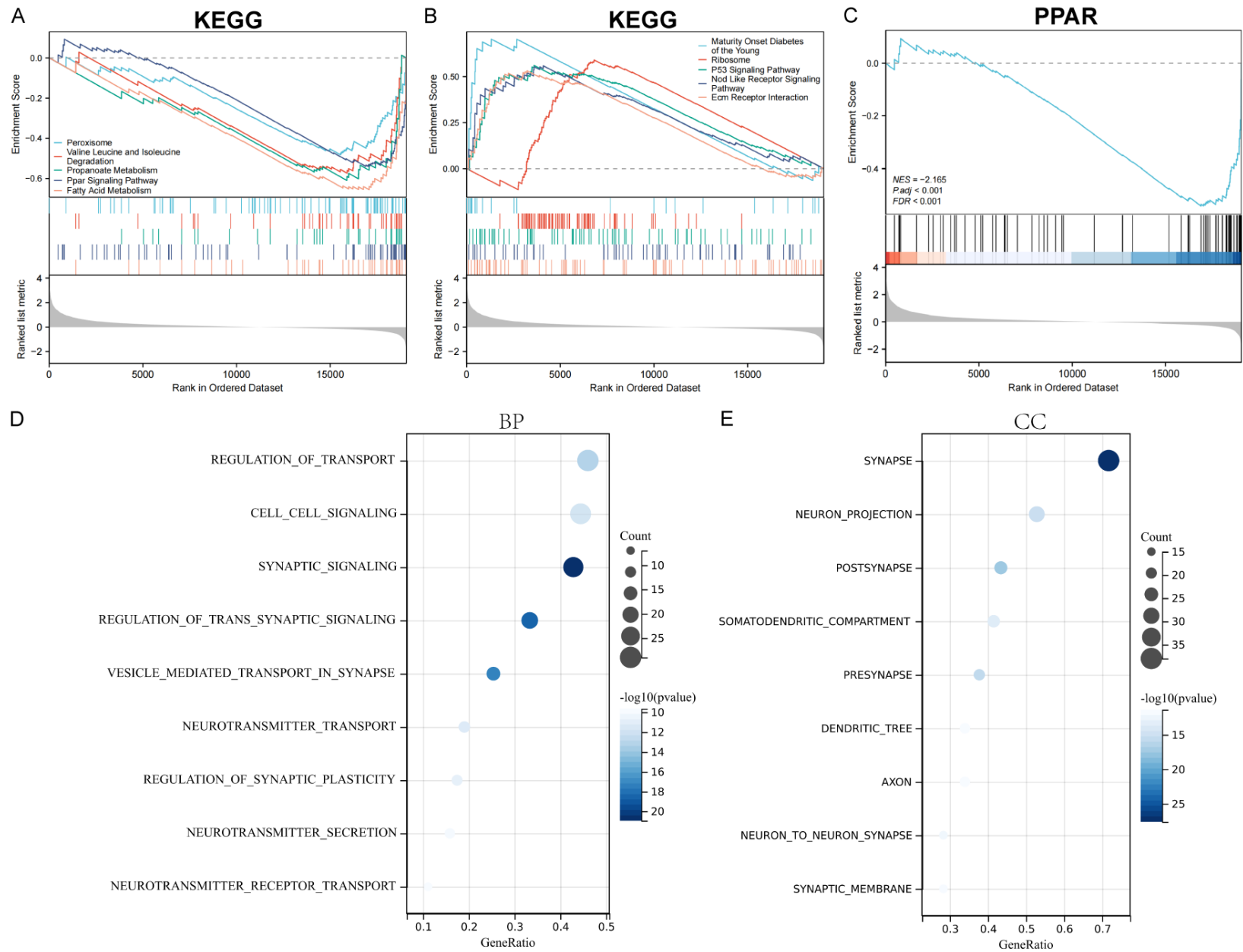


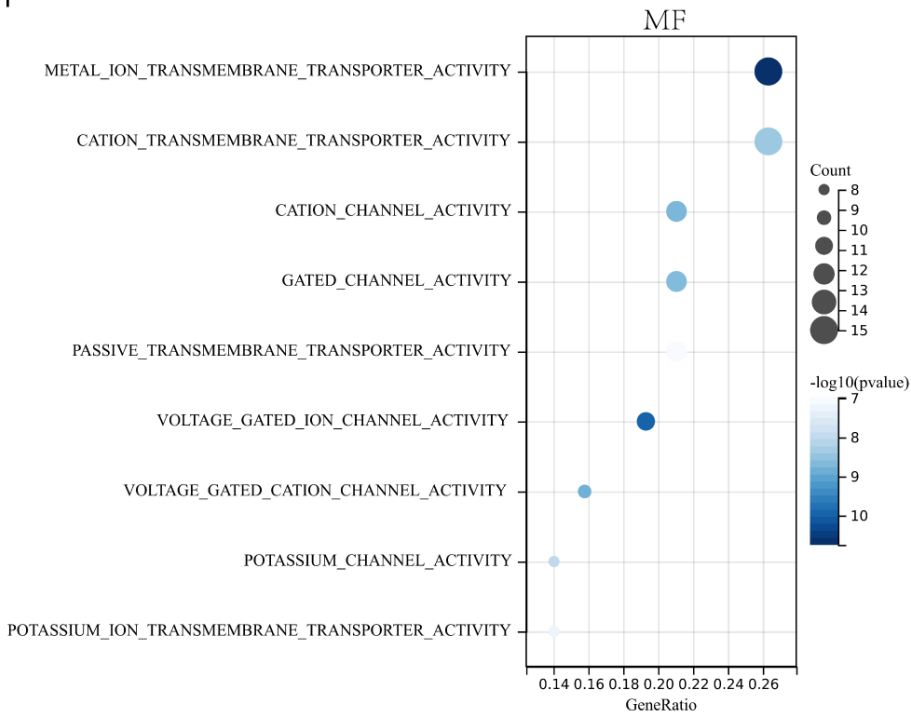
Figure 3. Co-expression analysis and pathway analysis of ITPKA. A. Co-expressed gene network of ITPKA; B. Protein-protein interaction (PPI) network diagram of ITPKA; C. Expression heatmap of the top 100 ITPKA-related genes; D. Heatmap showing clinical correlations between ITPKA co-expressed genes and tumor-node-metastasis (TNM) stage and pathological grade of KIRC patients.

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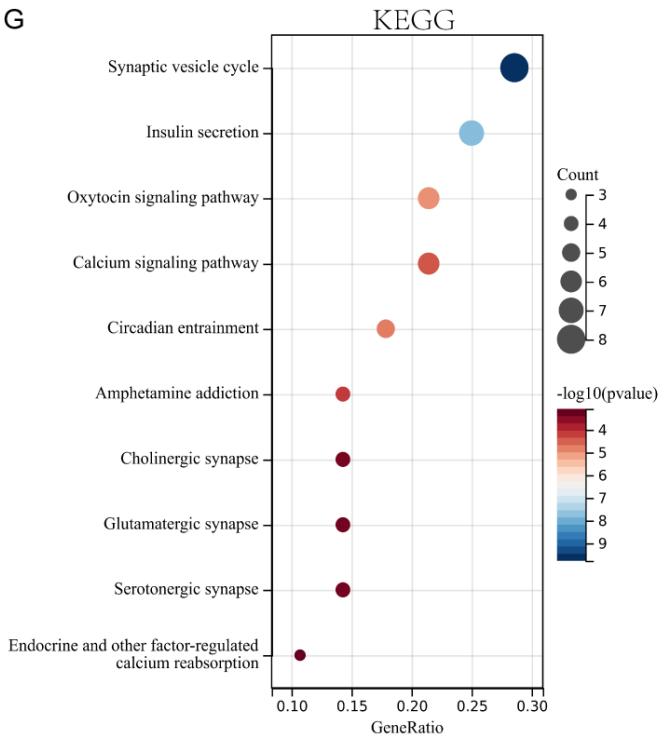


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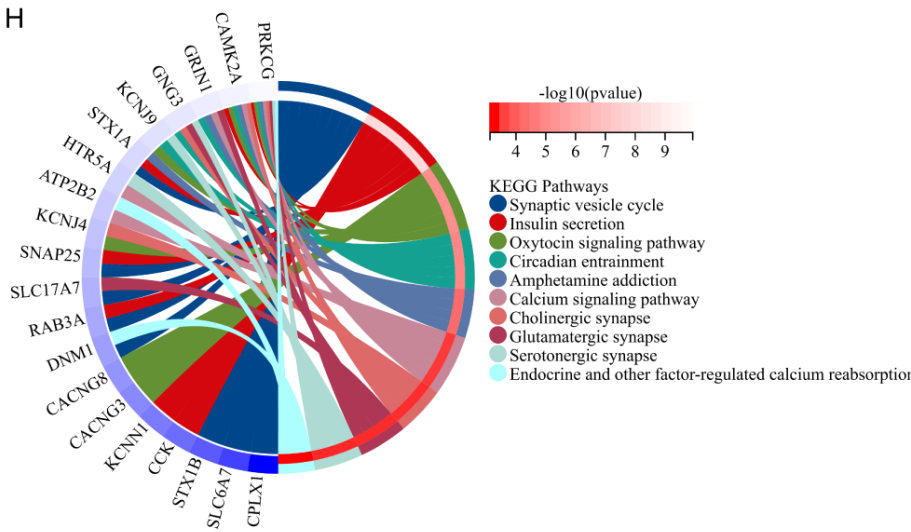
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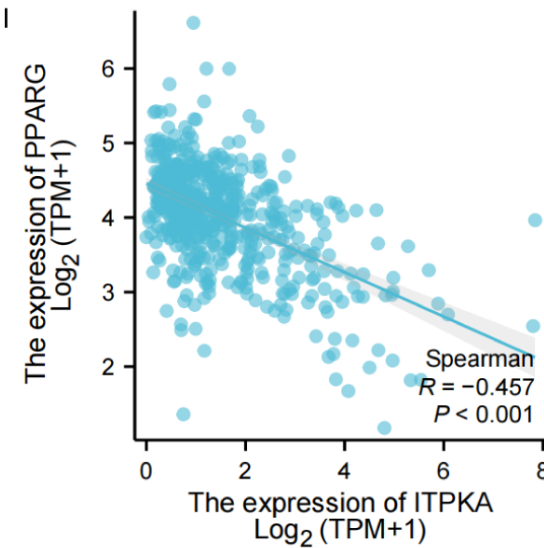


Figure 4. Functional enrichment analysis of ITPKA-related differentially expressed genes in TCGA-KIRC cohort. A, B. KEGG pathway enrichment analysis, showing the top positively and negatively enriched pathways associated with ITPKA-related differential expression; C. PPAR signaling pathway enrichment in ITPKA low-expression conditions; D-F. GO enrichment analysis of ITPKA-associated differentially expressed genes; G, H. KEGG enrichment analysis of ITPKA-associated differentially expressed genes; I. ITPKA expression shows the strongest negative correlation with PPARG among the examined PPAR isoforms ($R = -0.457$). Notes: KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology.

tive control group (**Figure 7A, 7D**). Transwell assays further showed that ITPKA knockdown significantly inhibited both migration and invasion capacities of 786-O cells (**Figure 7B, 7E, 7F**). Western blot analysis showed that ITPKA knockdown increased the expression of PPAR pathway-related proteins, particularly PPARG and PPARG (**Figure 7C, 7G**).

PPAR γ -targeted rescue assays support a functional role of PPAR γ -related signaling in ITPKA-associated malignant phenotypes

PPAR γ -targeted rescue assays showed that pharmacological inhibition of PPAR γ with GW9662 partially restored the migration and invasion phenotypes suppressed by ITPKA knockdown, whereas PPARG overexpression attenuated the malignant phenotypes induced by ITPKA overexpression. In wound healing assay, the migration rate of ITPKA-knockdown cells (KD + Vehicle) was significantly reduced compared with that of the negative control cells (NC + Vehicle); and this reduction was partially rescued upon treatment with the PPAR γ antagonist GW9662. Conversely, forced PPARG expression attenuated the enhanced migratory capacity associated with ITPKA overexpression (OE + Vector) (**Figure 8A**). Consistently, CCK-8 proliferation assays revealed that ITPKA knockdown markedly suppressed 786-O cell proliferation, an effect partially attenuated by GW9662 co-treatment. In contrast, ITPKA overexpression (OE + Vector) promoted proliferation, whereas co-expression of PPARG counteracted this pro-proliferative effect (**Figure 8B**). Transwell migration and invasion assays further confirmed these trends: the number of transmembrane cells decreased significantly in the KD + Vehicle group compared with NC + Vehicle group; GW9662 treatment partially restored transmigration capacity; and co-expression of PPARG in ITPKA-overexpressing cells (OE + PPARG-OE) significantly reduced transmembrane cell counts compared with OE + Vector controls (**Figure 8C**). At the protein level, ITPKA knockdown upregulated PPARG, PPARG, and

E-cadherin expression while downregulating vimentin expression; GW9662 treatment partially reversed these changes. Moreover, PPARG overexpression in the OE + PPARG-OE group normalized the dysregulated expression of PPAR pathway components and epithelial-mesenchymal transition (EMT) markers induced by ITPKA overexpression (**Figure 8D, 8E**). These findings support a functional association between ITPKA expression and PPAR pathway activity in 786-O ccRCC cells, although underlying biochemical mechanism remains unresolved. Future studies are needed to determine whether ITPKA directly phosphorylates PPAR isoforms or their co-factors, or indirectly modulates their nuclear translocation, protein stability, or transcriptional activity through downstream pathways such as calcium signaling.

Discussion

RCC is a common malignancy of the kidney, with ccRCC exhibiting poorer survival outcomes compared to other RCC subtypes [25, 26]. Imbalance of multiple metabolites is closely associated with the development and progression of ccRCC [27]. Identification of metabolism-related molecular markers is crucial for early detection, prognostic prediction, and development of targeted therapeutic strategies in ccRCC.

In this study, ITPKA was highly expressed in ccRCC and significantly elevated in metastatic cases, suggesting a potential role in disease progression. IHC analysis of 20 paired ccRCC and adjacent non-tumor tissues was performed as exploratory validation at protein level. This paired design allowed us to examine whether ITPKA protein expression followed the same trend as the transcriptomic findings from the TCGA-KIRC cohort. However, because of the limited sample size, these IHC data were not used to draw definitive associations between ITPKA protein expression and clinicopathological features or survival outcomes. Therefore, clinical relevance should be interpreted mainly

ITPKA as a biomarker in clear cell renal cell carcinoma

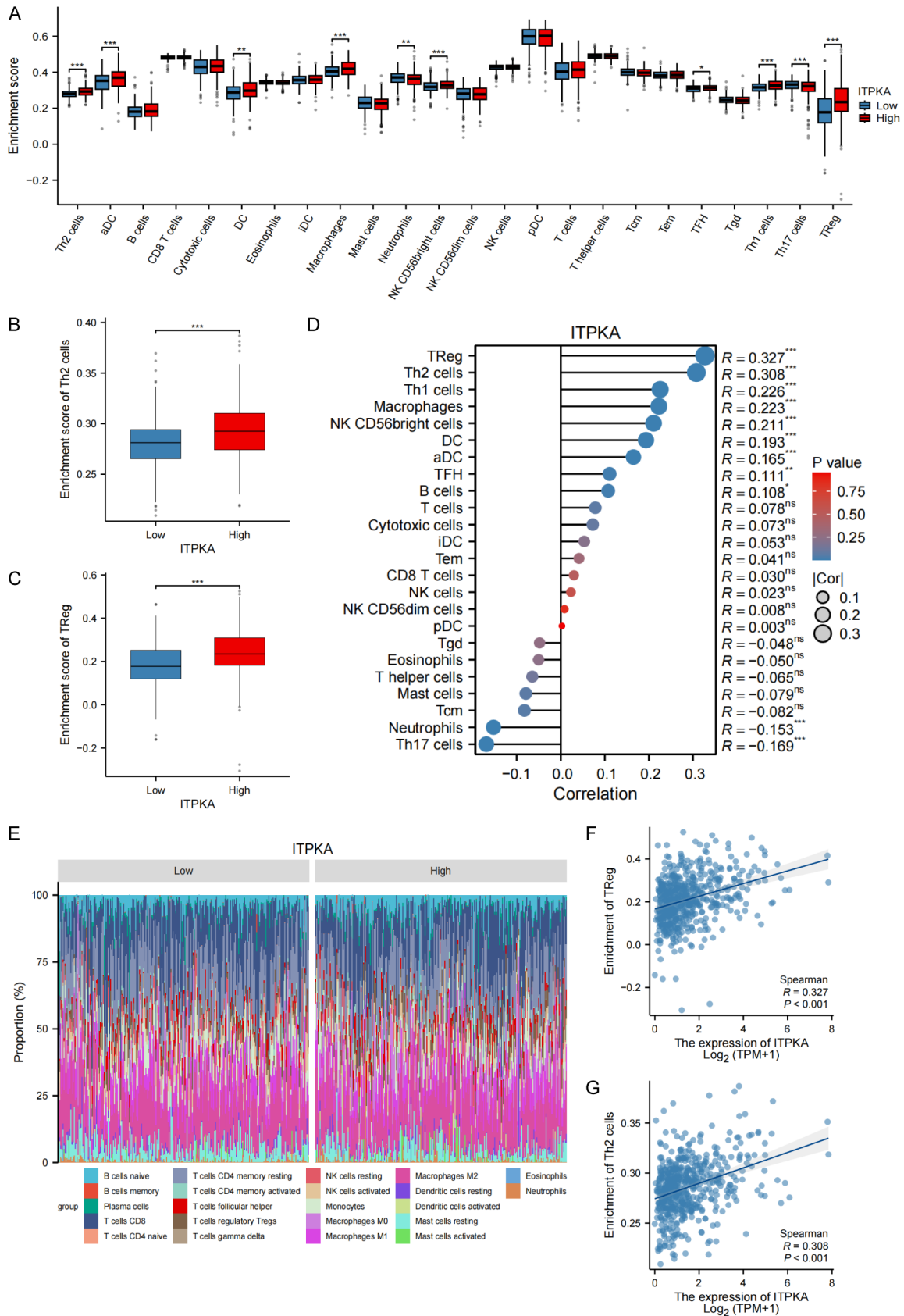


Figure 5. Association between ITPKA expression and immune cell infiltration. A. Comparison of enrichment scores for 24 immune cell populations between the high- and low- ITPKA-expression groups, presented as box plots; B, C. Comparisons of estimated enrichment scores for Th2 cells and Treg cells between the ITPKA high- and low-expression groups ($P < 0.001$); D. Bubble plot illustrating the correlation between the estimated infiltration scores of 24 immune cell populations and ITPKA expression; E. Heatmap showing the correlation between ITPKA expression levels and immune cell infiltration scores across various immune cell types; F, G. ITPKA expression levels were positively correlated with Th2 cell and Treg cell enrichment scores. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

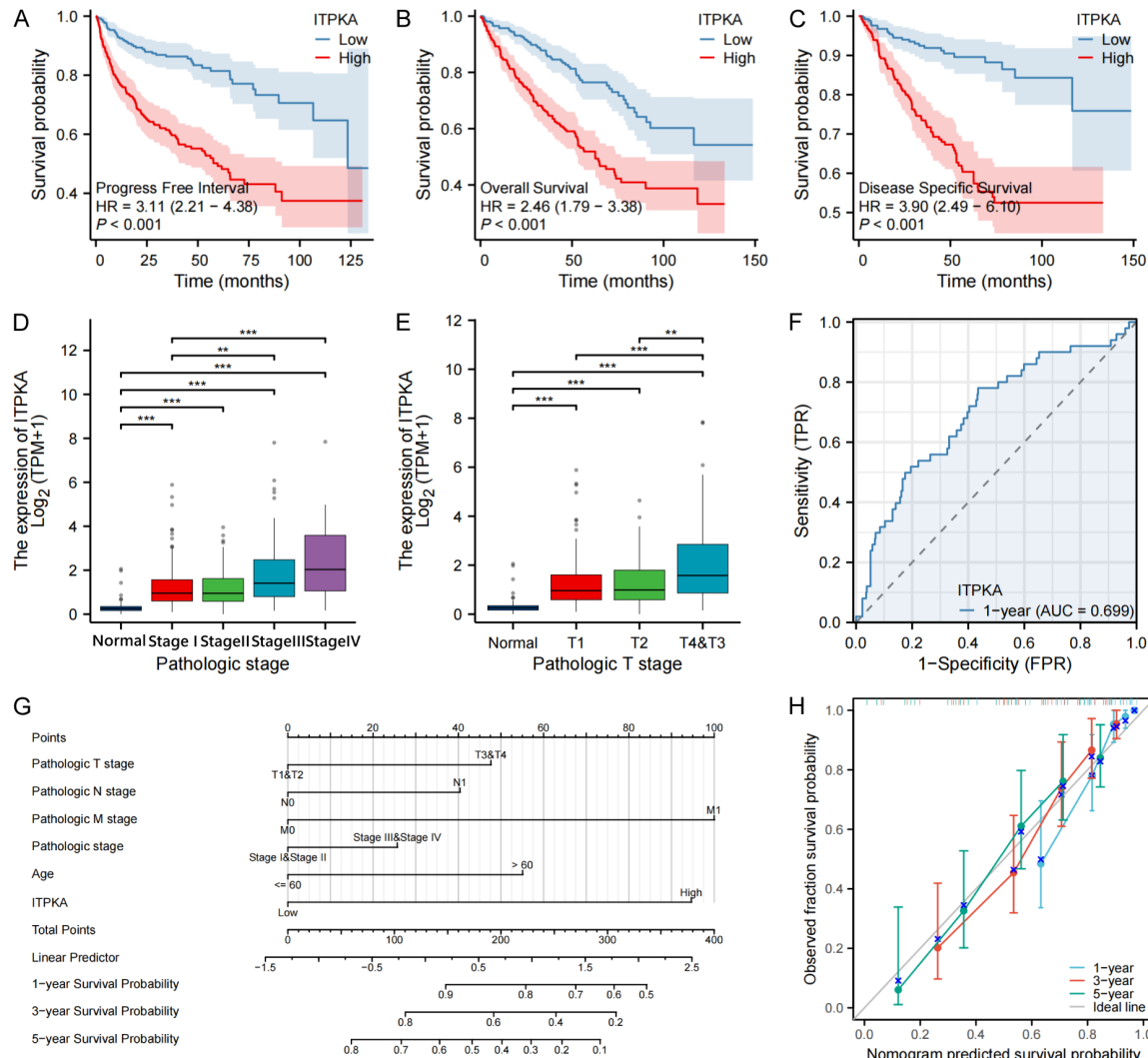


Figure 6. Relationship between ITPKA expression and clinicopathological characteristics in the TCGA-KIRC cohort. A-C. Kaplan-Meier survival analysis of PFI, OS, and DSS between the ITPKA low- and high-expression group in KIRC cohort; D, E. Relationship between ITPKA expression and pathological grade and TNM stage. F. ROC curve evaluating the prognostic predictive performance of ITPKA in KIRC. G. Prognostic nomogram for predicting 1-, 3-, and 5-year OS. H. Calibration curves assessing the agreement between predicted and observed 1-, 3-, and 5-year overall survival probabilities. Notes: PFI, progression-free interval; OS, overall survival; DSS, disease-specific survival; ROC, receiver operating characteristic. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

from the larger TCGA-KIRC dataset, with IHC results providing preliminary orthogonal support.

In addition, we identified several genes co-expressed with ITPKA in the occurrence and

development of ccRCC, including PLCB1, INPP5A, CAMK2, and CALMs through PPI network analysis. Among them, phospholipase C b1 (PLCB1) has been associated with hyperactive disorders, such as schizophrenia, epileptic encephalopathy, and myotonic dystrophy [28],

Table 2. Clinical baseline information characteristics and their association with ITPKA expression in ccRCC patients from the TCGA-KIRC cohort

Characteristics	Low expression of ITPKA	High expression of ITPKA	P value
n	270	271	
Pathologic T stage, n (%)			<0.001
T1	111 (20.5%)	168 (31.1%)	
T2	37 (6.8%)	34 (6.3%)	
T3	113 (20.9%)	67 (12.4%)	
T4	9 (1.7%)	2 (0.4%)	
Pathologic N stage, n (%)			0.302
N0	119 (46.1%)	123 (47.7%)	
N1	10 (3.9%)	6 (2.3%)	
Pathologic M stage, n (%)			<0.001
M0	208 (40.9%)	221 (43.5%)	
M1	55 (10.8%)	24 (4.7%)	
Pathologic stage, n (%)			<0.001
Stage I	107 (19.9%)	166 (30.9%)	
Stage II	29 (5.4%)	30 (5.6%)	
Stage III	74 (13.8%)	49 (9.1%)	
Stage IV	58 (10.8%)	25 (4.6%)	
Primary therapy outcome, n (%)			0.037
SD	1 (0.7%)	5 (3.4%)	
PR	0 (0%)	2 (1.4%)	
CR	45 (30.6%)	83 (56.5%)	
PD	8 (5.4%)	3 (2%)	
Gender, n (%)			0.006
Male	192 (35.5%)	162 (29.9%)	
Female	78 (14.4%)	109 (20.1%)	
Age, n (%)			0.366
≤60	129 (23.8%)	140 (25.9%)	
>60	141 (26.1%)	131 (24.2%)	
Histologic grade, n (%)			<0.001
G1	1 (0.2%)	13 (2.4%)	
G2	98 (18.4%)	138 (25.9%)	
G3	112 (21%)	95 (17.8%)	
G4	56 (10.5%)	20 (3.8%)	
Serum calcium, n (%)			0.515
Low	105 (28.6%)	99 (27%)	
Normal	81 (22.1%)	72 (19.6%)	
Elevated	7 (1.9%)	3 (0.8%)	
Hemoglobin, n (%)			0.112
Low	151 (32.8%)	113 (24.5%)	
Normal	91 (19.7%)	101 (21.9%)	
Elevated	3 (0.7%)	2 (0.4%)	
Laterality, n (%)			0.438
Left	131 (24.3%)	122 (22.6%)	
Right	139 (25.7%)	148 (27.4%)	

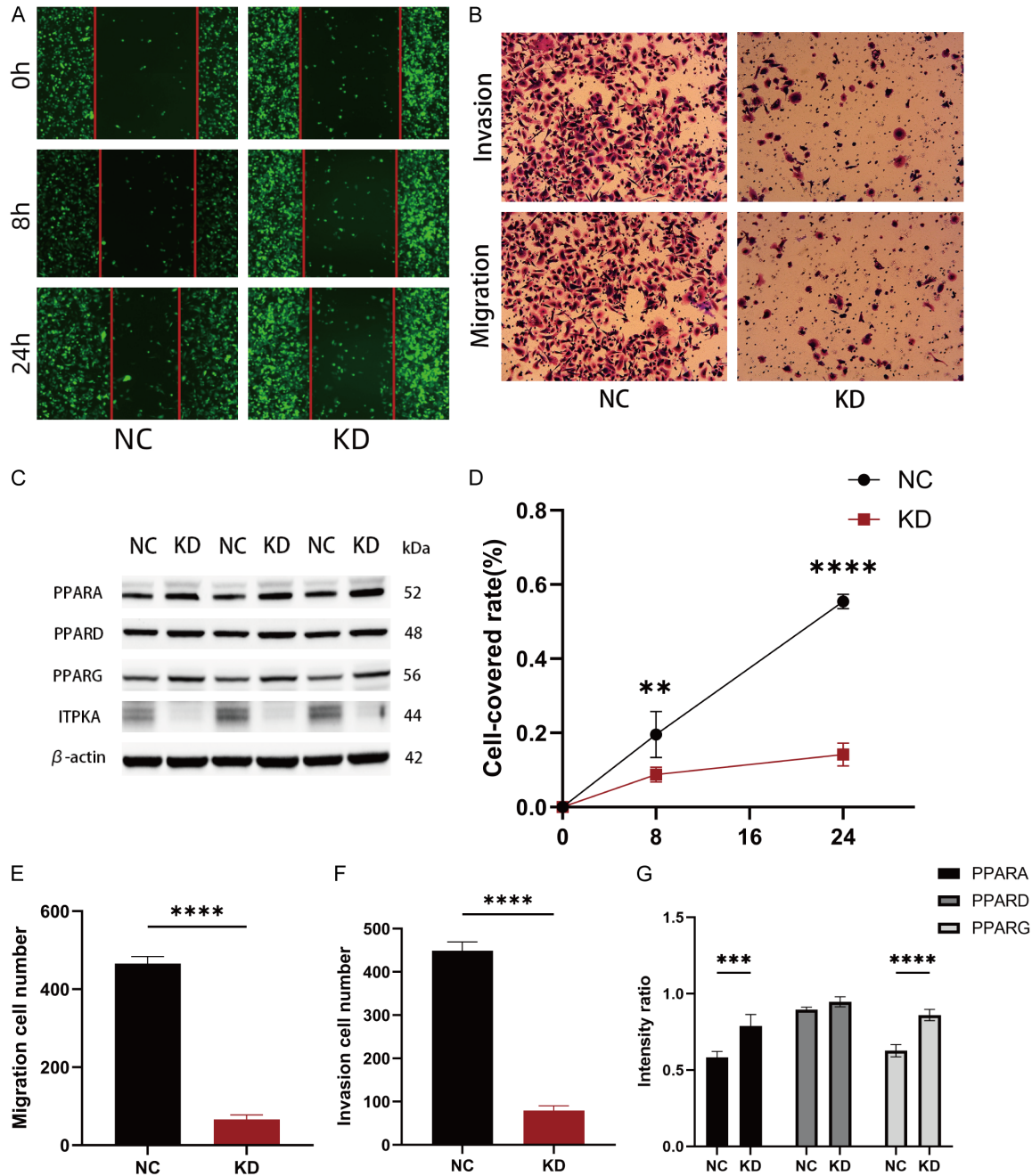


Figure 7. ITPKA knockdown suppresses migration and invasion of 786-O cells and modulates PPAR pathway-related protein expression. A, D. Wound-healing assays showing that ITPKA knockdown significantly reduced tumor cell migratory capacity ($\times 100$ magnification); B, E, F. Transwell assays demonstrating that ITPKA knockdown significantly inhibited cell invasion and migration abilities; C, G. Western Blot assays showing that ITPKA knockdown altered the expression of PPAR pathway-related proteins. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

and is also involved in cell cycle regulation and proliferation, acting as a tumor-initiating factor in small-cell lung, breast, and colorectal cancers [29]. These findings suggest that ITPKA may be functionally associated with PLCB1-related signaling networks in ccRCC, although

the regulatory mechanisms require further experimental validation. INPP5A has been implicated in melanoma and esophageal carcinogenesis [30, 31]; aberrant INPP5A expression can lead to intracellular inositol trisphosphate (IP3) accumulation, overactivation of IP3

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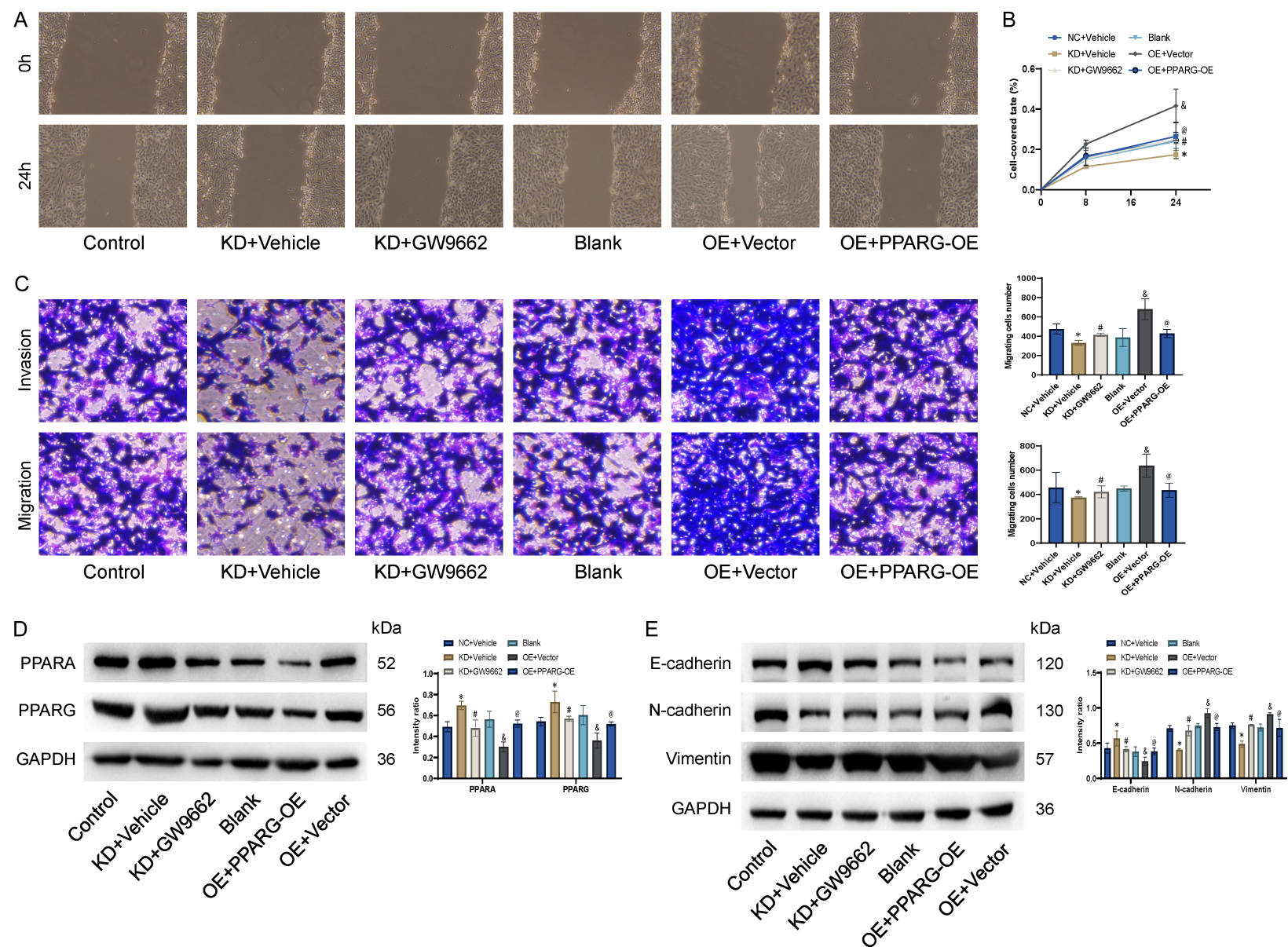


Figure 8. PPAR γ -targeted rescue assays indicate that PPAR γ inhibition partially reverses ITPKA knockdown-suppressed malignant phenotypes in 786-O cells. A. Wound-healing assays showing the migratory capacity of ccRCC cells in different groups. Representative images ($\times 100$ magnification) and quantitative analysis of

wound closure rate at 24 h. B. CCK-8 assays depicting proliferation curves of cells in each group at 0, 8, and 16 h. C. Transwell migration and invasion assays showing the number of transmembrane cells ($\times 200$ magnification). D. Representative Western blot images of PPARA, PPARG, E-cadherin, Vimentin, and GAPDH (loading control) in each group. E. Quantitative analysis of relative protein expression levels normalized to GAPDH. * $P < 0.05$ vs control group, # $P < 0.05$ vs KD+Vehicle group, & $P < 0.05$ vs blank group, @ $P < 0.05$ vs OE+PPARG-OE group.

receptors, and enhanced p53-dependent apoptosis, as well as modulation of tumor cell proliferation. Calcium/calmodulin-dependent protein kinase 2 (CAMK2) is highly expressed in triple-negative breast cancer and is involved in ferroptosis inhibition, thereby reducing the efficacy of immune checkpoint blockade therapy (ICB) [32]. Members of the calmodulin family (CALMs) are associated with neurodevelopment [33–36] and play roles in the development of steatohepatopathies and pancreatic cancers, suggesting a synergistic function with ITPKA in metabolic regulation. These bioinformatic results indicate that ITPKA may contribute to tumor-promoting pathways in ccRCC, but further experimental studies are required to elucidate the precise downstream network.

To explore the molecular mechanisms associated with ITPKA in tumor growth, functional enrichment analysis was performed. The results showed that ITPKA participates in biological processes such as intercellular signaling, neurotransmitter release, molecular transmembrane transport, ionic channel activity, insulin secretion, and calcium signaling. Calcium signaling, a dynamic process, plays an important role in both normal cellular activities and pathological conditions. Calcium ions modulate actin participates dynamics through calmodulin and calpain, thereby facilitating epithelial-mesenchymal transition (EMT) and enhancing tumor cell motility. S100 calcium-binding proteins secreted by ccRCC (e.g., S100A9) promote distant metastasis by recruiting immunosuppressive cells and degrading the extracellular matrix [37]. Moreover, calcium ions influence mitochondrial function and key glycolytic enzymes, influencing cellular energy metabolism. Calcium overload can induce immunogenic cell death (ICD) in tumor cells, releasing damage-associated molecular patterns (DAMPs) that activate T-cell responses. However, within the acidic tumor microenvironment, $\text{Ca}^{2+}/\text{H}^{+}$ interactions recruit myeloid-derived suppressor cells (MDSCs), contributing to an immunosuppressive barrier. Calcium signaling also strictly regulates gene transcription,

proliferation, neovascularization, and the metabolic programming of immune cells [38], thereby remodeling the tumor microenvironment. Taken together, these findings suggest that calcium-related signaling may provide a biologically plausible context for ITPKA-mediated modulation of tumor cell behavior. However, whether ITPKA functionally links calcium signaling to PPAR pathway activity in ccRCC remains to be experimentally clarified.

Metabolic reprogramming is recognized as a hallmark features of tumorigenesis and development [39]. In the present study, GSEA results indicated that ITPKA-associated genes were significantly enriched in metabolism-related pathways. Among them, dysregulation of the PPAR signaling pathway, which plays a key role in fatty acid metabolism, glucose metabolism, and amino acid degradation, may contribute to metabolic reprogramming that support rapid tumor proliferation and adaptation [40]. PPARs form heterodimers with retinoic acid X receptor (RXR), which then bind to peroxisome proliferator-activated receptor elements (PPRE) within the promoter regions of target genes. Through interactions with transcriptional co-activators or co-repressors, PPAR-RXR complexes regulate the expression of genes involved in lipid metabolism, energy homeostasis, and tumor-associated metabolic processes [18, 41]. Dysregulation of the PPAR signaling pathway may confer metabolic advantages that promote rapid tumor proliferation and enhance cellular aggressiveness [42, 43].

The tumor microenvironment (TME) is an important determinant of tumor progression and therapeutic responsiveness [44, 45]. In this study, transcriptomic deconvolution analysis suggested that ITPKA expression was associated with estimated infiltration levels of several immune cell subsets, particularly Treg and Th2 cells [46]. However, these findings should be interpreted as hypothesis-generating rather than experimentally validated evidence of immune regulatory function. Bulk RNA-seq-based immune infiltration algorithms cannot deter-

mine the spatial distribution of immune cells, define direct cell-cell interactions, or identify the cellular source of ITPKA expression within tumor tissues. Therefore, we avoided inferring that ITPKA directly recruits or activates specific immune cell populations. Further validation using multiplex immunohistochemistry, spatial transcriptomics, or single-cell RNA sequencing will be necessary to clarify whether ITPKA expression is mechanistically associated with immune cell composition and immunotherapeutic responsiveness in ccRCC.

We investigated the prognostic value of ITPKA by analyzing its correlation with clinicopathological features in the TCGA-KIRC cohort. ITPKA expression was closely correlated with TNM classification and pathological stage. Elevated ITPKA expression was correlated with advanced tumor stage, as well as increased likelihood of lymph node involvement and distant metastasis, suggesting an association with unfavorable clinical outcomes in ccRCC patients. Survival analyses showed that patients with high ITPKA expression had significantly shorter OS, PFI and DSS. The results of subgroup prognostic analysis were consistent.

Multivariate Cox regression analysis was also utilized to establish a prognostic nomogram incorporating independent prognostic variables. Calibration analysis showed close agreement between predicted and observed 1-, 3-, and 5-years OS probabilities, indicating satisfactory predictive performance of the model. This suggests that the nomogram developed in this study may serve as a potentially useful tool for prognostic assessment in ccRCC.

To further verify the biological role of ITPKA in ccRCC, functional experiments were performed *in vitro*. ITPKA knockdown markedly suppressed the proliferation, migration, and invasion of 786-O cells. Notably, all functional experiments were conducted in 786-O cells, a widely used VHL-deficient ccRCC model. Although this model is relevant to ccRCC biology, it cannot fully capture the molecular heterogeneity of ccRCC. Therefore, these findings should be interpreted as preliminary evidence derived from a single *in vitro* ccRCC model rather than definitive evidence broadly applicable to all ccRCC contexts. Further validation using additional ccRCC cell lines, patient-derived models, and *in vivo* experimental systems will

be necessary to determine the generalizability of the ITPKA-associated phenotypes.

In addition, Western blot analysis showed that ITPKA knockdown altered the expression of several PPAR pathway-related proteins, suggesting that PPAR signaling may participate in ITPKA-associated cellular phenotypes. However, these protein-level changes should not be interpreted as evidence of a direct biochemical regulation between ITPKA and PPAR pathway components.

In this study, PPAR-targeted rescue assays further suggested that PPAR pathway activity contributes to ITPKA-associated malignant cellular phenotypes. Pharmacological inhibition of PPAR γ partially reversed the suppressive effects of ITPKA knockdown, whereas PPARG overexpression attenuated the pro-migratory and pro-invasive phenotypes associated with ITPKA overexpression. These findings support a functional relationship between ITPKA expression and PPAR pathway activity in 786-O cells. However, the current experiments do not establish a direct biochemical interaction between ITPKA and PPAR pathway components. Therefore, the term “PPAR pathway inhibition” should be interpreted as functional suppression at the pathway level rather than direct kinase-substrate regulation. Whether ITPKA directly interacts with PPAR γ , phosphorylates PPAR-related cofactors, or indirectly modulates PPAR transcriptional activity through calcium signaling, cytoskeletal remodeling, or other upstream events remains to be clarified. These results raise the possibility that PPAR pathway activation, particularly PPAR γ -related signaling, may contribute to suppression of malignant phenotypes in ccRCC models. Notably, several PPAR γ agonists and related compounds have already been clinically approved for metabolic disorders. These findings provide a preliminary rationale for further investigating whether PPAR-related pharmacological strategies may have therapeutic relevance in ccRCC characterized by high ITPKA expression. However, this possibility remains exploratory and requires further validation in additional cell models, animal studies, and clinically annotated cohorts.

PPAR agonists have demonstrated significant therapeutic efficacy in treating several metabolic diseases. Among these, β -type drugs (PPAR α agonists) and thiazolidinediones

(PPAR γ agonists) are widely used in the treatment of dyslipidemia, type 2 diabetes, and prevention of cardiovascular and cerebrovascular diseases, representing two major classes of PPAR-targeted therapeutics [41]. Given the involvement of PPAR β/γ signaling in processes related to tumor biology, including oxidative stress regulation, tumor-associated macrophage polarization, angiogenesis, and epithelial-mesenchymal transition, further investigation of PPAR-targeted strategies in cancer remains biologically relevant. Notably, our study provides functional evidence that ITPKA-associated malignant phenotypes are accompanied by alterations in PPAR pathway activity. However, the current data do not establish a direct molecular interaction between ITPKA and PPAR signaling components. One plausible and testable hypothesis is that ITPKA, by phosphorylation Ins(1,4,5)P₃ to Ins(1,3,4,5)P₄, modulates intracellular calcium homeostasis, thereby influencing PPAR γ phosphorylation, nuclear translocation, or transcriptional activity. Alternatively, ITPKA may interact with and phosphorylate PPAR γ co-regulators to suppress its transcriptional function. Definitive validation of these hypotheses will require additional mechanistic studies, including co-immunoprecipitation assays to assess protein-protein interactions, *in vitro* kinase assays to assess direct phosphorylation, and PPAR-responsive luciferase reporter assays to measure transcriptional activity.

Taken together, the present study provides preliminary evidence that ITPKA is associated with ccRCC progression and functionally linked to PPAR γ -related signaling *in vitro*. However, these findings should be interpreted with caution, as the IHC validation cohort, immune infiltration analysis, PPAR γ rescue experiments, and single-cell-line functional assays all require further validation.

Limitations

Several limitations of the present study should be acknowledged. First, the IHC cohort included only 20 paired ccRCC and adjacent tissue samples. This cohort was designed as an exploratory protein-level validation set and was not designed or powered to establish robust associations between ITPKA protein expression and clinicopathological features or survival outcomes. Therefore, the prognostic relevance

of ITPKA should primarily be interpreted based on the larger TCGA-KIRC analysis and requires further validation in independent multicenter cohorts. Second, the immune infiltration results were derived from computational deconvolution of bulk transcriptomic data. Although these analyses suggested that ITPKA expression may be associated with specific immune cell infiltration patterns, they cannot confirm immune cell abundance with spatial localization, or determine direct immunoregulatory interactions at the tissue level. Additional studies using multiplex immunohistochemistry, spatial transcriptomics, and single-cell RNA sequencing will be necessary to validate the relationship between ITPKA expression and the tumor immune microenvironment. Third, although PPAR-targeted rescue assays supported a functional relationship between ITPKA and PPAR pathway activity, the present study did not demonstrate a direct biochemical interaction between ITPKA and PPAR pathway components. Further mechanistic investigations, including co-immunoprecipitation, *in vitro* kinase assays, PPAR-responsive luciferase reporter assays, and subcellular localization studies, are required to clarify whether ITPKA directly regulates PPAR γ activity or indirectly influences PPAR signaling through upstream processes such as calcium signaling or cytoskeletal remodeling. Finally, all *in vitro* experiments were performed using the 786-O cell line. While 786-O is a commonly used ccRCC model, the observed findings may be influenced by cell-line-specific features. Future validation using additional ccRCC cell lines, patient-derived models, and *in vivo* systems are necessary to determine the broader applicability of these findings.

Conclusion

This study suggests that ITPKA is upregulated in ccRCC and may be associated with tumor progression and unfavorable prognosis. Exploratory IHC analysis provided preliminary protein-level support for increased ITPKA expression in ccRCC tissues, while transcriptome-based immune infiltration analysis suggested a potential association between ITPKA expression and the tumor immune microenvironment. In 786-O cells, ITPKA knockdown suppressed malignant cellular phenotypes, and PPAR γ -targeted rescue experiments suggested that inhibition of PPAR γ partially restored the malignant phenotypes suppressed by ITPKA knock-

down, supporting a functional association between ITPKA expression and PPAR γ -related signaling. However, the direct biochemical mechanism linking ITPKA to PPAR signaling and the immunological relevance of ITPKA require further validation. These findings provide preliminary evidence supporting ITPKA as a potential biomarker and functional regulator in ccRCC, while highlighting the need for larger clinical cohorts and additional experimental models.

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Written informed consent was obtained from all participants before specimen collection.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Hai Zhu, Department of Urology, Qingdao Municipal Hospital, Qingdao University, No. 1 Jiaozhou Road, Shibei District, Qingdao 266000, Shandong, China. Tel: +86-15505326680; E-mail: fyluyongguo@sdsu.edu.cn

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