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Identifying TYMP as an Immune Prognostic Marker in Clear Cell Renal Cell Carcinoma

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Abstract

Background: In clear cell renal cell carcinoma (ccRCC), only some patients can benefit from immunotherapy therapy, and it is urgent to find immune-related molecular markers and targets. **Methods:** Thymidine phosphorylase (TYMP) expression level and predictive value in pan-cancers were analyzed using TIMER, GEPIA2, and The Human Protein Atlas. We obtained ccRCC tissues to verify the differential expression of TYMP and confirmed the biological function in vitro. Subsequently, Gene Ontology, Kyoto Encyclopedia of Genes and Genomes (KEGG), and Gene Set Enrichment Analysis (GSEA) are used to explore the potential mechanism of TYMP. Finally, TIMER was used to analyze the infiltration levels and prognostic value of different immune cells. **Results:** TYMP is upregulated in various cancers, including ccRCC, and there is a certain degree of causality between high expression and poor prognosis in ccRCC. It was confirmed that TYMP knockdown could suppress cell aggressiveness, and cause cell death. Differential analysis showed that 55 differential genes were upregulated in the high-expression groups of TYMP. KEGG and GSEA analyses suggested that TYMP was linked to immune cell invasion, fatty acid metabolism, and P53 signaling pathway. Further investigation revealed that the expression level of TYMP linked positively to T-cell follicular helper and Tregs, but negatively with mast cell activation. Finally, a Nomogram was established on the base of expression level of TYMP and the clinical characteristics of ccRCC patients to predict prognosis. **Conclusions:** Patient survival is poor and immune cell infiltration is abnormal when TYMP is highly expressed in ccRCC, suggesting that ccRCC patients could benefit from using TYMP as a molecular diagnostic and therapeutic target.

Keywords

TYMP, ccRCC, immune regulation, nomogram, bioinformatics analysis

Abbreviations

CCL5, C-C Motif Chemokine Ligand 5; CTLA4, Cytotoxic T-Lymphocyte Associated Protein 4; CXCL9, C-X-C Motif Chemokine Ligand 9; CXCL13, C-X-C Motif Chemokine Ligand 9; FC, Fold Change; GEPIA, Gene Expression Profiling Interactive Analysis; LAG3, Lymphocyte Activating 3; PCR, Polymerase Chain Reaction; PDCD1, Programmed Cell Death 1; P53, Tumor Protein 53; TIMER, Tumor Immune Estimation Resource; VEGFA, Vascular Endothelial Growth Factor A.

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Introduction

Renal cancer is very common in urological malignancies, the incidence is high in both male and female patients.[1] The proportion of malignant renal tumors was significantly higher than that of benign ones.[2] A good 5-year survival rate can be achieved in early or localized renal cell carcinoma (RCC), with the help of partial or radical nephrectomy. Due to radiation and chemotherapy resistance, surgical excision remains the optimal treatment for RCC.[3] Patients with advanced renal cancer, even receive nephrectomy, are prone to metastases.[4] Despite the fact that clear cell RCC (ccRCC)-targeted treatment has some effect, the prognosis for individuals with advanced renal cancer remains poor.[5,6] On the basis of clinical data, ccRCC accounts for more than 70% of renal carcinoma.[7] To develop successful treatment methods for patients with renal malignancies, a full understanding of the

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mechanisms connected to ccRCC formation and the screening of biomarkers and targets of ccRCC is urgently required.

Thymidine phosphorylase (TYMP) was once referred to as endothelial cell growth factor-1 (ECGF1). TYMP was previously reported as platelet-derived angiogenic activity; however, recently discovered to be linked to TYMP, whose action is dependent on thymidine against thymidine catalysis by 2-deoxy-d-ribose, which is an active driver of endothelial responses.[8] TYMP is closely associated with tumor angiogenesis, including non-small cell lung cancer, Paget's disease, ductal breast cancer, and a variety of cancers of the digestive system including gastric cance.[8] In some tumors, strong expression of VEGFA is associated with increased microvessel density and poor prognosis.[9,10] TYMP metabolites can participate in cell metabolism and signaling pathways, playing a regulatory role in cell apoptosis and DNA synthesis.[11] However, no studies have demonstrated differential TYMP expression in ccRCC and its biological function. Using bioinformatics and tissue validation, this research determined that the levels of TYMP mRNA expression and protein were elevated in ccRCC and were related to bad prognosis for patients. Functional studies confirmed that TYMP affects the aggressiveness of renal cancer cells. Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Set Enrichment Analysis (GSEA) analyses also found a close correlation between TYMP and immune infiltration, fatty acid metabolism, and the P53 signaling pathway in ccRCC patients. Further investigation revealed a favorable correlation between TYMP and regulatory T cells (Tregs), which regulate the prognosis of ccRCC patients. Therefore, TYMP may be an immune target relevant to the early diagnosis and prognosis of ccRCC patients, with significant potential value.

Materials and Methods

Differential Expression Analysis

TIMER web services platform (https://cistrome.shinyapps.io/timer/) can be utilized as a systemic analysis tool for many types of cancer immunity infiltration.[12,13] TIMER has 7 functional modules for various bioinformatics analyses. The Diff Exp module investigates gene expression differences between tumor and normal tissue. Using boxplots to display the distribution of gene expression levels and the Wilcoxon test to determine the statistical significance of differential expression, we evaluated the differential expression of TYMP in pan-cancer utilizing this module. $P < .05$ were considered significant.

Standard processing activities on RNA sequencing expression data of cancer and healthy samples from the The Cancer Genome Atlas (TCGA) and GTEx projects are carried out on the GEPIA2 website (http://gepia2.cancer-pku.cn/#index).[14] Differential expression analysis of tumors and normal tissues, patient survival analysis, identification of genes with similar functions, correlation analysis, dimensionality reduction analysis was only few of the many applications for GEPIA2. GEPIA2 was used to examine the differential expression of TYMP in ccRCC and its relationship to the clinical stage. The cutoff value for absolute Log2FC was 1 and *P*-value was .05.

Every protein found in human cells has been mapped owing to Human Protein Atlas (https://www.proteinatlas.org/), tissues, and organs. TYMP protein expression in renal cancer and The Human Protein Atlas was utilized to analyze the protein content of normal tissues.[15]

The principal ccRCC cancers' gene expression patterns and TCGA provided the associated clinical information.[16] After correcting the data, differential analysis was performed with the "limma" R package. The threshold of differential gene screening was set as $|\log FC| > 1$ and adjusted $P < .05$.

Prognostic Analysis of TYMP

Using the GEPIA2 website, the prognosis of TYMP in pan-cancer and ccRCC was examined. The Group Cutoff was median, and $P < .05$ were considered significant.

Gene Ontology Analyses, Kyoto Encyclopedia of Genes and Genomes Analyses, and Gene Set Enrichment Analysis

The 'clusterProfile' R program was used to investigate the functional categories of differentially expressed genes using the Gene Ontology (GO) and KEGG databases. As a computational approach, GSEA can predict if the defined gene set exhibits statistical differences between 2 biological situations.[17] All genes in both the high and low TYMP expression groups were examined GSEA employing the "clusterProfile" R package. The GSEA analysis involved in this study was retrieved from the Molecular Signature Database (H: hallmark gene sets, gene symbols) (https://www.gsea-msigdb.org/gsea/msigdb/collections.jsp).

Immune Infiltration Analysis

The immune infiltration patterns of all TCGA tumors were retrieved from the website TIMER (http://timer.cistrome.org/). CIBERSORT is a potent analytical instrument that employs a gene expression signature comprised of 547 genes.[18] It identifies each subtype of immune cell and uses a deconvolution technique to precisely quantify unique immune cell compositions (CCs). Immune infiltration data were downloaded from TIMER, and immune infiltrating cells assessed using CIBERSORT method were screened from TCGA-kidney renal clear cell carcinoma (KIRC) cohort. The infiltrating expression levels of various immune cells in renal cell cancer and adjacent tissues were studied and compared. Using the "survival" R package, the predictive significance of different immune cell infiltration levels was examined. Patients with $a > 30$ days survival time were screened for prognostic analysis using the Cox proportional hazards regression model and Survival analysis (Log-Rank test). $P < .05$ was used as the cut-off value. Then, we investigated the amount of immune cell infiltration between the 2 TYMP expression groups, and the connection between TYMP and immune cell invasion.

Nomogram

The R tool "rms" was utilized to estimate the overall survival (OS) of KIRC patients at 1, 3, and 5 years in the TCGA cohort based on the clinical features of ccRCC patients and the expression level of TYMP. Consequently, a prognostic nomogram was built, and a calibration curve was developed to assess the prognostic performance of the nomogram.

Collection of Clinical Specimens

A total of twenty ccRCC tissue specimens and paracancer tissue specimens were consecutively collected between January 2022 and December 2022. These patients were pathologically diagnosed with ccRCC and underwent surgical resection at the Department of Urology, Shandong Provincial Hospital Affiliated with Shandong First Medical University (Jinan, China). Before surgery, none of the patients received any anticancer therapy. All patients and participants' family members provided their written consent for study participation. We had de-identified all patient details. The study protocol was preapproved by the Ethics Committee of the Shandong Provincial Hospital Affiliated with Shandong First Medical University (NO.SWYX2023-239). The reporting of this study conforms to REMARK guidelines.[19]

Cell Culture

The National Certified Cell Culture Collection supplied normal human renal tubular epithelial cells (HK-2) and 786-O. HK-2 and 786-O cells were cultured in an incubator containing 5% CO_2 and RPMI 1640 medium enriched with 10% fetal bovine serum (Thermo Fisher Scientific) and 1% penicillin-streptomycin (Sigma Aldrich) at 37 °C.

RNA Isolation and Real-Time PCR

Utilizing the TRIzol reagent (Invitrogen), total RNA was extracted from various cells and tissues. and converted to cDNA according to the manufacturer guidelines employing PrimeScript^{TM} RT Master Mix (Takara). Real-time PCR using the SYBR-Green (Takara) kit was employed to measure the mRNA expression levels of the main predictive gene TYMP. The following is the order of the primers used in this study: Internal reference β-actin forward: 5′-CACAGCAAGAGAGGCATCC-3′; reverse: 5′-CTGGGG TGTTGAAGGTCTC-3′. TYMP forward: 5′-CTGCTGTAT CGTGGGTCAGAGT-3′; reverse: 5′-TACTGAGAATGGAGGC TGTGATG-3′.

Cell Viability Assay

Using Cell Counting Kit-8 (Dojindo) according to the manufacturer guidelines, cell viability was determined. The 786-O cells were planted at 3000 cells per well in 96-well plates and cultivated for 24 h at 37 °C in a humidified incubator with 5% CO_2 and a temperature of 37 °C. After adding the interfering RNA for 24 h, the CCK-8 reagent was supplied again and the incubation was extended for 2 hours. At 450 nm, an OD value was determined using a microplate reader. A total of 3 repetitions of the experiment were conducted, once by each group.

Detection of Apoptosis by Flow Cytometry

In total, 48 h following transfection, the 786-O cells were recovered and digested with 0.25% trypsin. According to the instructions of Apoptosis Detection Kit (BD Biosciences), we resuspended the cells in pre-cooled 1x PBS (4 °C), centrifuged for 5-10 min at 2000 rpm, and then washed. Before analysis, $(5\text{-}6) \times 10^5$cells were combined with 500 μL of 1X Binding Buffer, 5 μL of Annexin-FITC, and 5 μL of PI in a dark environment at ambient temperature for 15 min.

Wound Healing

The 786-O cells transfected with NC-siRNA or TYMP-siRNA were plated at a density of 3×10^5 cells for every well in 6-well plates. When the cells achieved 100% confluence, a 200 μL pipette tip was utilized to draw 4 parallel vertical lines in each group of 7 replicate wells. The cells were cultivated for 24 h, and pictures were taken at 0 and 24 h. The migration of cells was monitored, and the distance of cell migration in each group was measured.

Statistics

R version 3.6.1 was employed for statistical analysis. The screening parameters for differential genes used in this study were $P < .05$ and logFC > 1. To assess the variations in survival between groups, Kaplan–Meier survival curves were built. To analyze group differences, the student's *t*-test was performed. Boxplots were made with the "ggboxplot" R package, heatmaps with the "pheatmap" R package, and line plots with the "ggscatter" R package. Results with $P < .05$ were considered significant.

Results

Differential Expression and Clinical Prognosis of TYMP in Solid Pan-Cancer

Figure 1A shows the differential expression of TYMP in bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical and endocervical cancer (CESC), cholangiocarcinoma (CHOL), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck cancer (HNSC), KIRC, kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), rectum adenocarcinoma (READ), rectum adenocarcinoma (STAD), uterine corpus endometrial carcinoma (UCEC), and pancreatic adenocarcinoma (PAAD). Results suggest that TYMP was elevated in all of the aforementioned malignancies with the exception of PAAD. GEPIA2 website study indicated that TYMP is connected to a negative prognosis in adrenocortical carcinoma (ACC), KIRC, brain lower grade

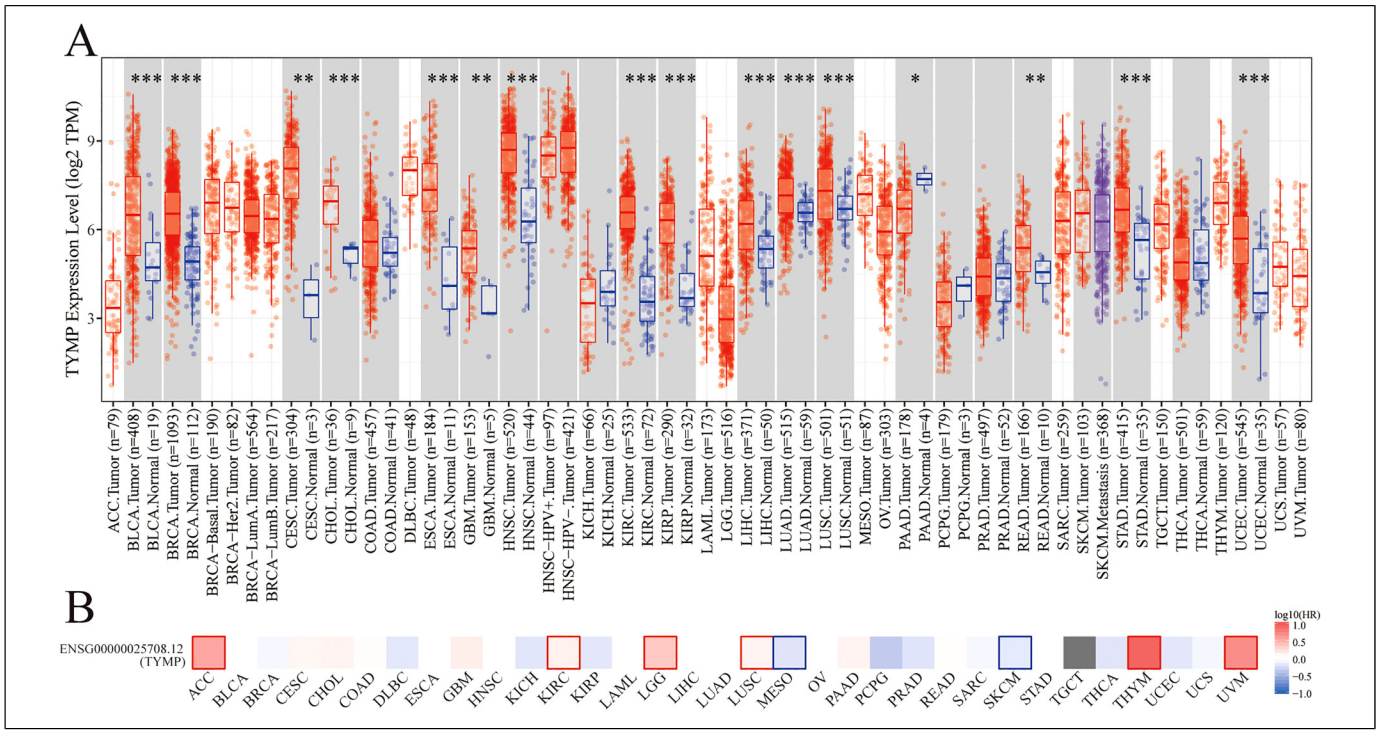

Figure 1. Differential expression and clinical prognosis of thymidine phosphorylase (TYMP) in pan-cancer. (A) TIMER website was utilized to evaluate differential TYMP expression in pan-cancer, *P*-value significant codes: $0 \leq *** < .001 \leq ** < .01 \leq * < .05$; (B) GEPIA2 was used to analyze TYMP prognostic value in pan-cancer.

glioma (LGG), LUSC, Thymoma (THYM), and uveal melanoma (UVM), but a favorable prognosis in mesothelioma (MESO) and Skin Cutaneous Melanoma (SKCM) (Figure 1B).

Differential Expression of TYMP in ccRCC

The differential expression and clinical value of TYMP mRNA in ccRCC were validated using GEPIA2. Figure 2A and B illustrates the upregulated mRNA expression of TYMP in ccRCC, this linked to a negative prognosis for patients. This study also discovered that the mRNA expression level of TYMP was different in ccRCC of different clinical stages—the higher the clinical stage, the higher the expression level of TYMP (Figure 2C). Finally, the level of TYMP protein expression in renal cancer and healthy renal tissues was investigated using data from the Human Protein Atlas website. Renal cancer tissue, as shown in Figure 2D, expresses more TYMP protein than does normal renal tissue.

Experimental Verification of Differential Expression and Biological Function of TYMP

RNA was taken from ccRCC and paracancer tissues, and differential expression of TYMP was investigated using PCR. According to the results of the study, ccRCC tissues express TYMP at a significantly higher rate than paracancer tissues do (Figure 3A). Moreover, TYMP expression level was significantly increased in 786-O renal carcinoma cells than in HK-2 cells (Figure 3B).

We constructed and synthesized interfering RNA and then transfect them into 786-O cells to investigate the biological function of TYMP. PCR was used to verify that the interfering RNA can significantly reduce the expression of TYMP, indicating their effectiveness for subsequent experiments to be carried out (Figure 3C). After TYMP knockdown in 786-O cells, the proliferation ability of the cells was significantly reduced, as indicated by CCK-8 detection (Figure 3D). Alternatively, Flow cytometry demonstrated a significant rise in the apoptosis rate of cells (Figure 3E). The migration ability of cells was also significantly reduced, as characterized by wound healing (Figure 3F). These findings imply that TYMP involves in controlling renal cancer cell aggressiveness.

Screening of TYMP-Related Differential Genes

The expression profile of the TCGA-KIRC cohort, which consists of 532 tumor and 72 normal tissues, was taken directly from the dataset. The tumor tissues from ccRCC patients were examined for further study. Affirmed by the computed median TYMP expression levels, ccRCC individuals were classified into high and low TYMP expression groups. Utilizing the "limma" R tool, differential genes between the 2 TYMP expression groups were identified. The analysis revealed the upregulation of 55 differential genes with the values of abs(logFC) > 1 and adjusted $P < .05$ (Figure 4A). These differentially expressed genes include PDCD1, LAG3, CXCL9, CXCL13, CCL5, and other immune-related genes. Figure 4B shows

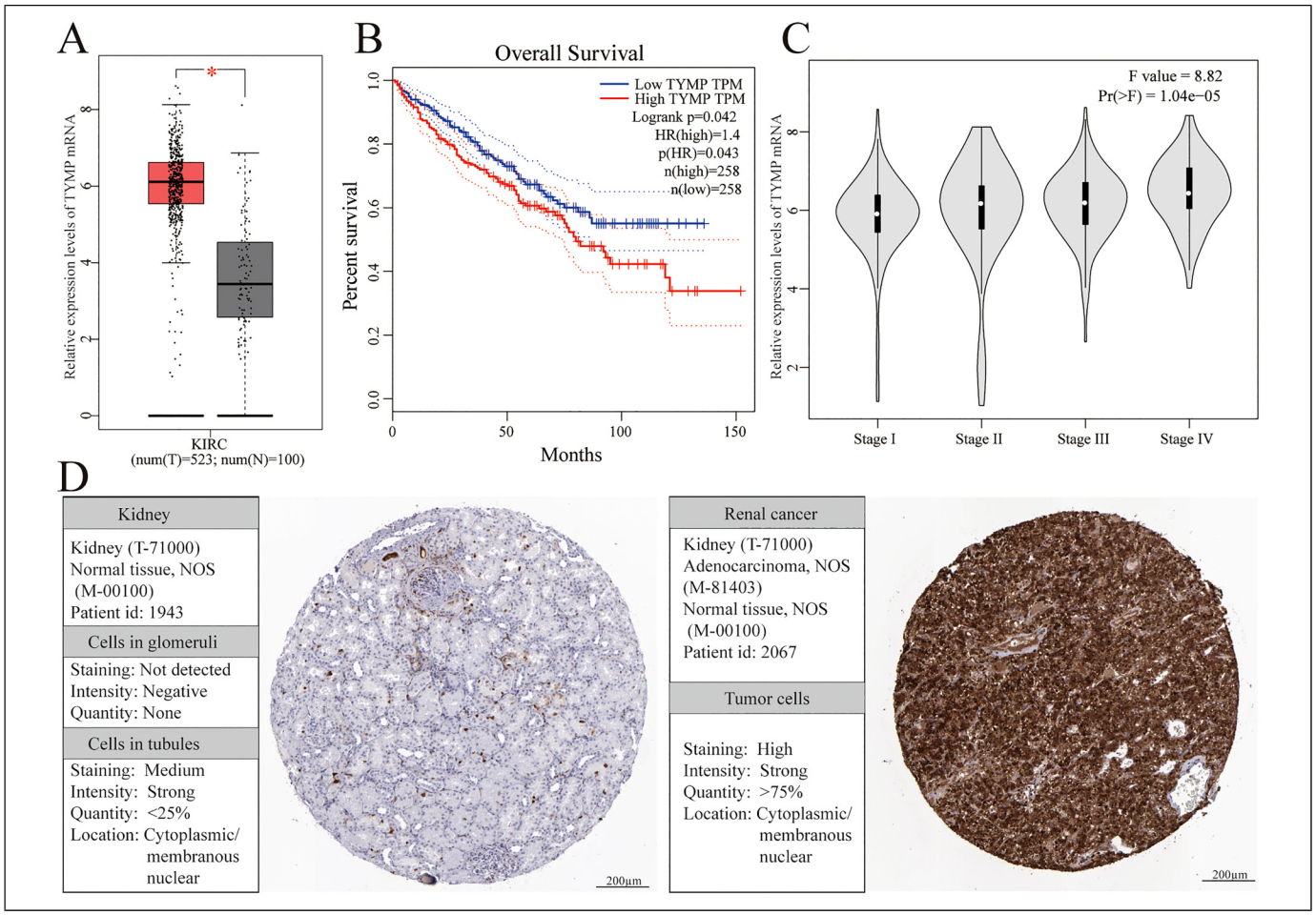

Figure 2. The clinical value of TYMP in ccRCC. (A) GEPIA2 was utilized for differential expression analysis of TYMP in ccRCC, where $P <$ .05 was judged as statistically significant. (B) GEPIA2 was utilized to review the predictive value of TYMP in ccRCC. (C) GEPIA2 was utilized for differential expression analysis of TYMP in ccRCC at different clinical stages. (D) Utilizing the Human Protein Atlas website, TYMP protein expression levels in renal cancer and normal renal tissues were analyzed. Abbreviations: TYMP, thymidine phosphorylase; ccRCC, clear cell renal cell carcinoma.

several gene expression levels in the 2 TYMP expression groups. We further discovered a positive correlation between TYMP expression and the levels of PD-1 and CTLA4 as shown in Supplemental Figure S1A. Specifically, in the high TYMP expression subgroup, there was a significant increase in the expression levels of PD-1 and CTLA4 as shown in Supplemental Figure S1B.

Explore the Potential Biological Functions of TYMP

On the basis of TYMP-related differential genes, GO and KEGG analyses were done to investigate the probable biological roles of TYMP. Figure 5A shows the biological processes (BP), molecular function (MF), and CC of TYMP-related differentially expressed genes. BP is composed mostly of lymphocyte-mediated immunity, humoral immune response, immunoglobulin-mediated immune response, and B cell-mediated immunity. The CC is composed of blood microparticles, the outside surface of the plasma membrane, and the T cell receptor complex. Antigen binding, receptor-ligand activity, receptor regulator activity, and cytokine activity constitute the majority of MF. Figure 5B shows a KEGG analysis of differential genes associated with TYMP. In cancer, these genes are mostly involved in cytokine-cytokine receptor interaction, PD-L1 production, and the PD-1 checkpoint pathway, as well as viral protein interaction with cytokine and cytokine receptor and the T-cell receptor signaling pathway.

Further GSEA analysis was performed on all genes in high and low TYMP expression groups. Figure 6A and B depicts the enrichment of multiple immune-related pathways, such as INTERFERON ALPHA RESPONSE, INTERFERON GAMMA RESPONSE, ALLOGRAFT REJECTION, and INFLAMMATORY RESPONSE. Other related signaling pathways also enriched include IL6 JAK STAT3 SIGNALING, P53 PATHWAY, and IL2 STAT5 SIGNALING.

Immune Cell Infiltration Related to ccRCC Prognosis

We obtained data from TIMER on the level of immune cell infiltration in the TCGA-KIRC cohort, filtered the data with

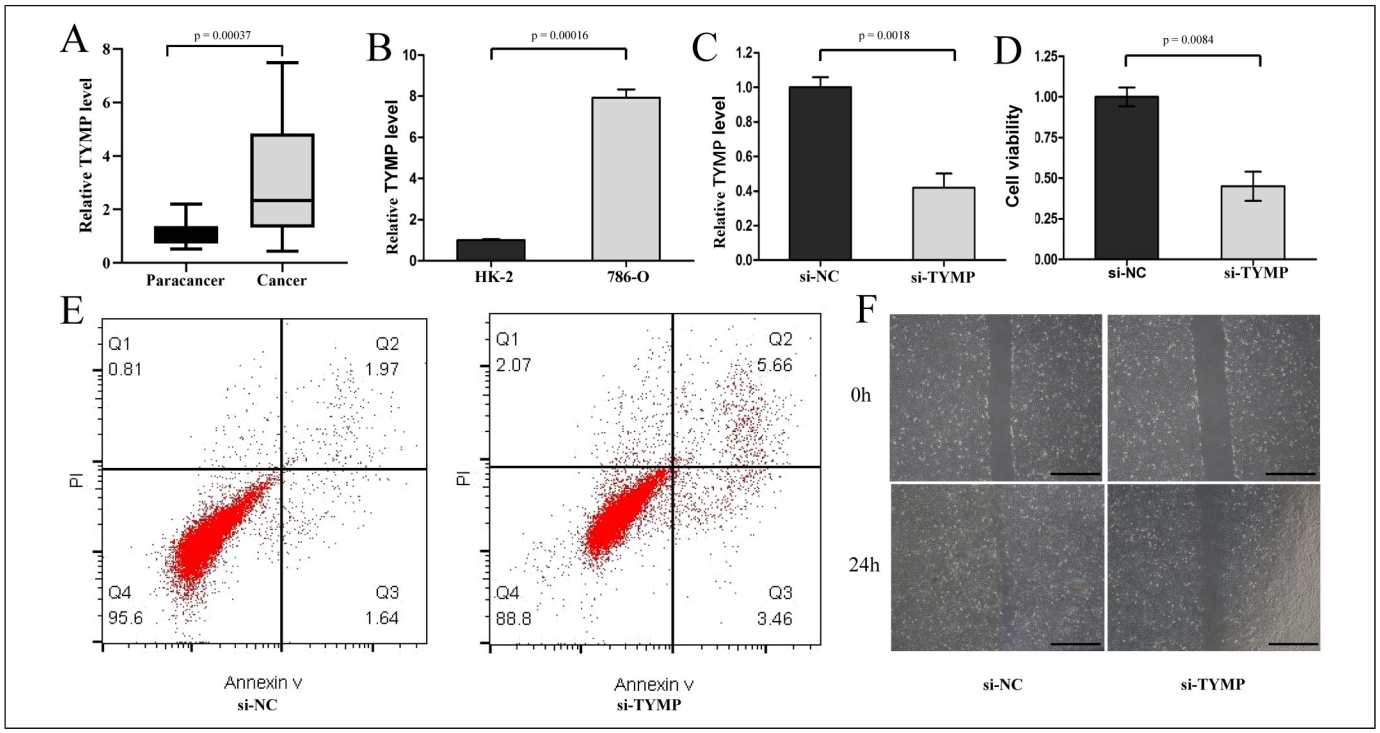

Figure 3. The differential expression and biological function of TYMP in ccRCC. (A) Differential expression of ATYMP in ccRCC and para cancer tissues. (B) Differential expression of TYMP in renal cancer cells (786-O cells) and normal renal tubular cells (HK-2 cells). (C) The interfering RNA can effectively knock down the expression of TYMP via PCR. (D) Proliferation capacity following TYMP knockdown was confirmed using a CCK-8 test. (E) After TYMP was knocked down, apoptosis could be detected using flow cytometry. (F) After TYMP was inhibited, cell migration was detected by observing wound healing (Scale bar, 200 μm, 40×). Abbreviations: TYMP, thymidine phosphorylase; ccRCC, clear cell renal cell carcinoma.

the CIBERSORT method to determine the amount of immune cell infiltration, and then performed further analysis. The infiltration levels of Macrophage M2, T cell CD8, Macrophage M0, Macrophage M1, T cell gamma delta, T-cell follicular helper, T-cell regulatory (Tregs), and resting NK cell were elevated in tumor tissue, as depicted in Figure 7A. In contrast, tumor tissue exhibited a decrease in the infiltration levels of T-cell resting CD4 memory and mast cell, activated myeloid dendritic cell, eosinophil, nascent B cell, active mast cell, monocyte, and B cell plasma. However, other immune cell infiltration levels were not significantly different.

Subsequently, we performed a prognostic analysis on immune cells. As displayed in Figure 7B, mast cell activation was related to a promising prognosis, whereas the infiltration levels of B cell memory, active T cell CD4 memory, T-cell follicular helper, T cell regulatory (Tregs), activated NK cell, and Macrophage M0 were linked to negative patient prognosis. Combined with differential analysis, the data demonstrate that T-cell follicular helper, Tregs, and Macrophage M0 with high infiltration levels in ccRCC are linked to negative patient prognosis. Also, mast cell activation with low infiltration levels leads to a poor patient prognosis. The results suggest that T-cell follicular helper, Tregs, Macrophage M0, and activated mast cells might have important roles in ccRCC.

Association Between TYMP Expression Level and Immune Cell Infiltration Level Related to Prognosis

To study further the immunological modulation of TYMP, the ccRCC patients were categorized into 2 groups based on median TYMP expression levels. In ccRCC patients, the connection between TYMP expression and prognosis-related immune cells was studied. As manifested in Figure 8A and B, in the high TYMP expression group, the infiltration levels of B cell memory, activated NK cell, T-cell follicular helper, and Tregs increased. In contrast, the infiltration level of the activated mast cell decreased. The degree of immune cell infiltration did not significantly vary with regard to prognosis. Following correlation analysis, Expression of T-cell follicular helper was observed to be significantly linked to expression of TYMP in ccRCC, Tregs, activated NK cell, and B-cell memory. A negative correlation with mast cell activation was also observed. Additionally, there was no correlation with other prognostic immune cells (Figure 8C).

Constructing a Predictive Model According to TYMP Expression Level and Clinical Characteristics

We combined TYMP expression levels with clinical features to create a nomogram to develop a quantitative method to assess

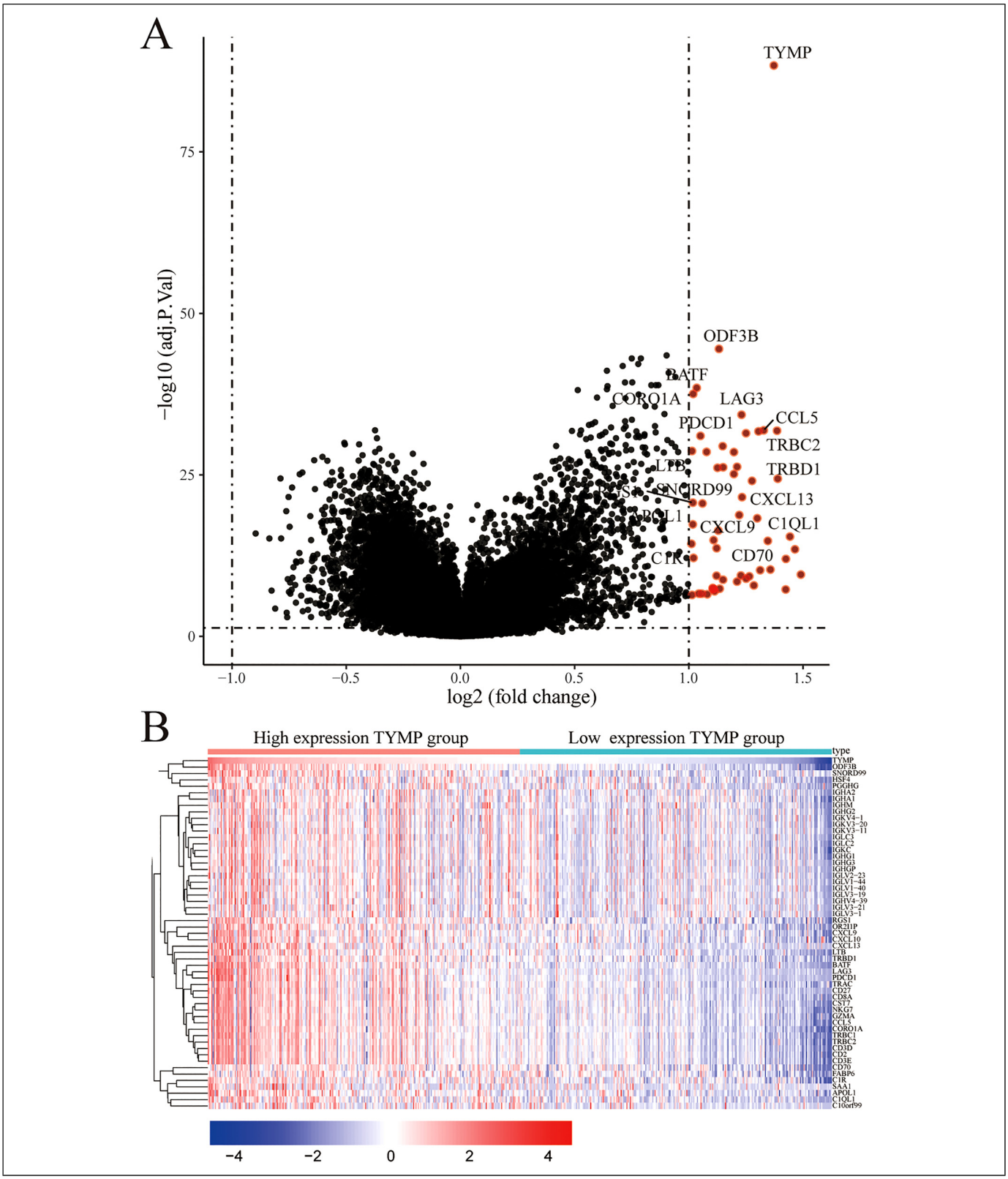

Figure 4. Differential genes associated with TYMP. TCGA-KIRC High- and low-TYMP patients were categorized. The volcano plot shows the differential genes in the 2 groups (A), and the heatmap illustrates the expression levels of the differential genes in the 2 groups (B). Abbreviations: TYMP, thymidine phosphorylase; TCGA, The Cancer Genome Atlas; KIRC, kidney renal clear cell carcinoma.

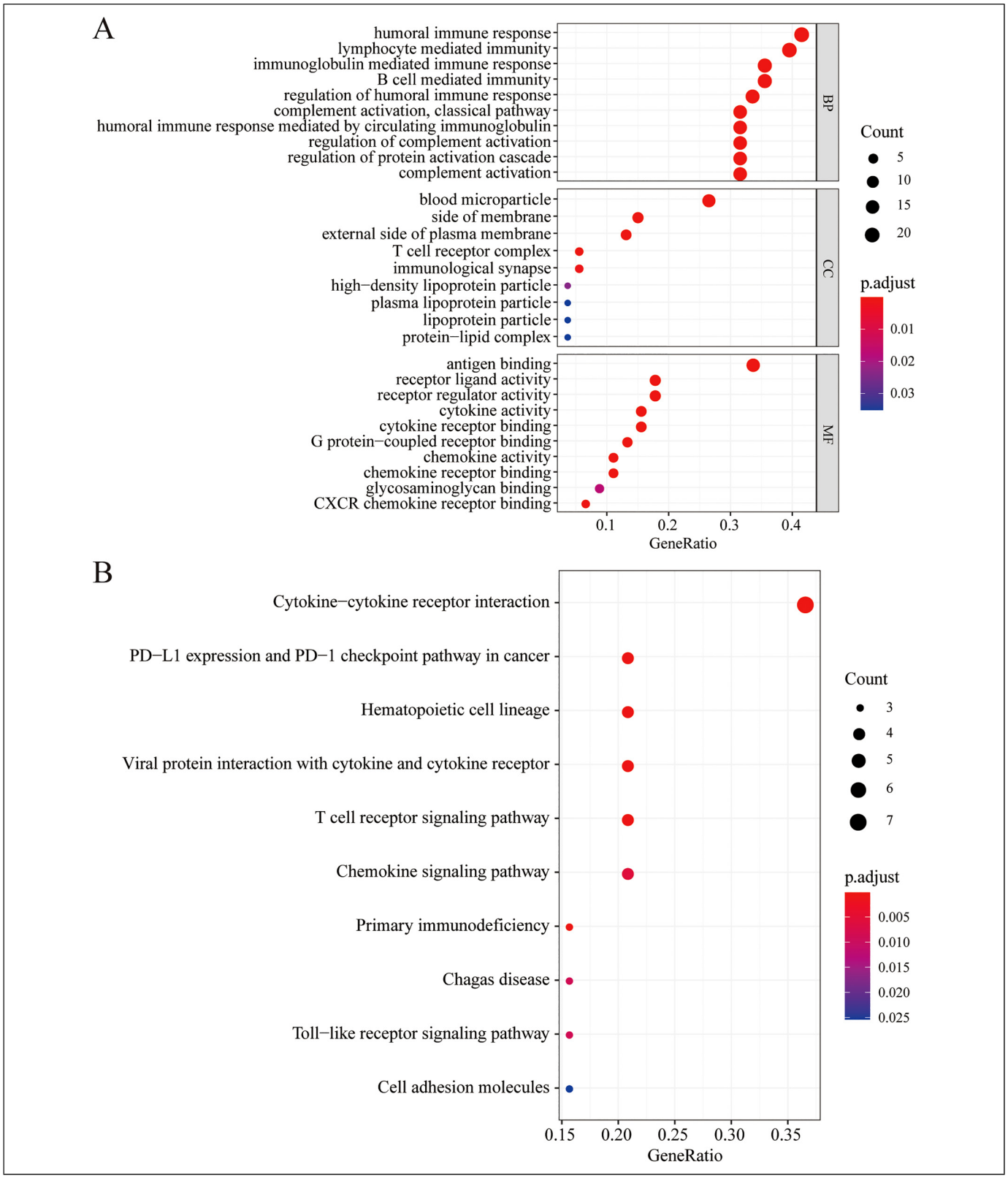

Figure 5. To study the potential biological functions of TYMP, GO, and KEGG studies were performed. (A) Involved in biological processes, cellular composition, and molecular function, genes with varying expression in the 2 TYMP expression groups were subjected to GO analysis. (B) Genes with high and low TYMP expression were compared utilizing KEGG analysis. Abbreviations: GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; TYMP, thymidine phosphorylase.

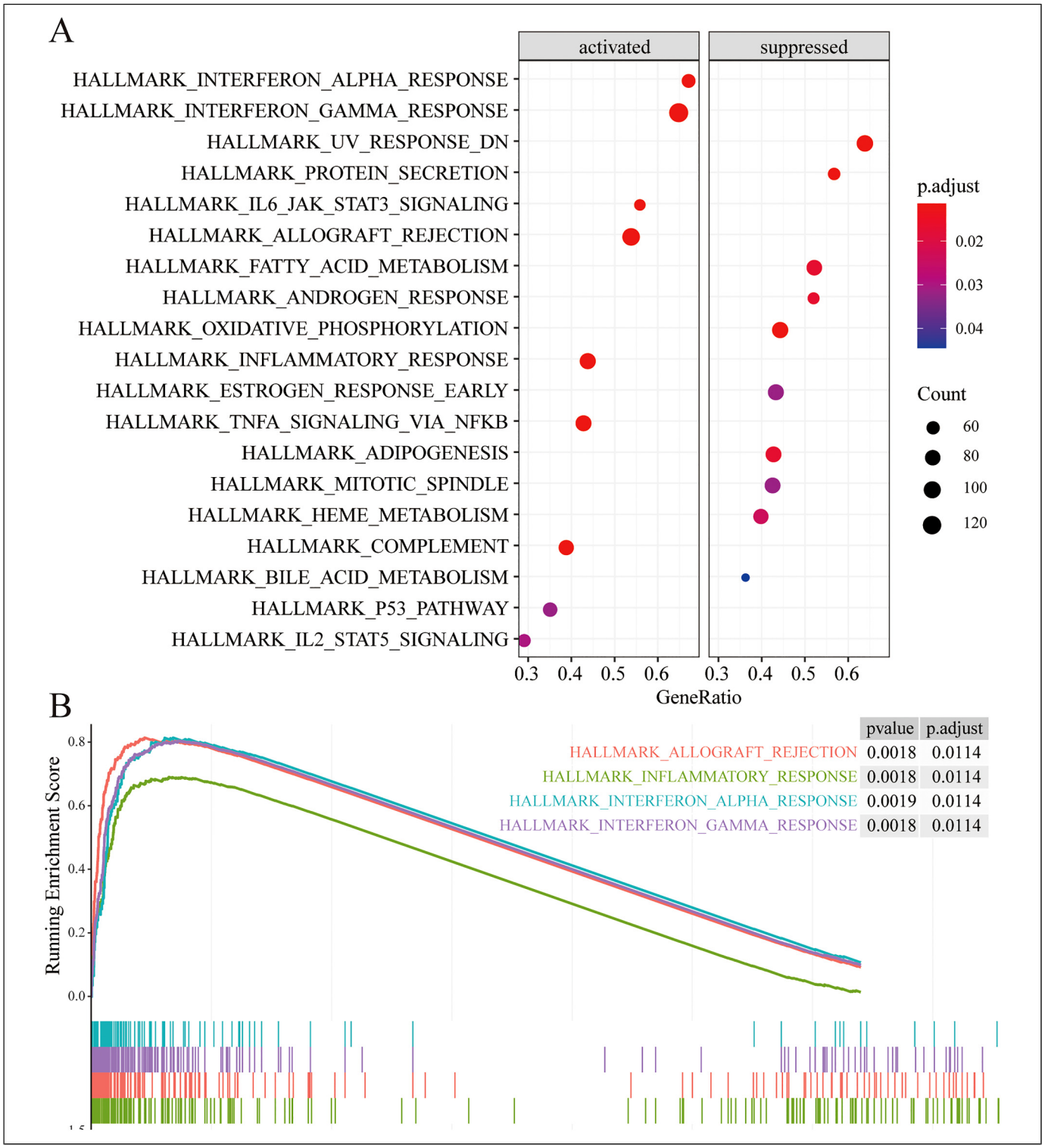

Figure 6. GSEA analysis of TYMP-related genes. (A) The bubble plot shows the relevant pathways enriched by GSEA. (B) Enrichment of some immune-related pathways. Abbreviations: TYMP, thymidine phosphorylase; GSEA, Gene Set Enrichment Analysis.

prognosis in ccRCC (Figure 9A). The patient's total score was calculated based on TYMP, age, gender, and pathologic stage. The patient's 1-, 3-, and 5-year survival was predicted based on the score to help practitioners participate in developing ccRCC patients. The nomogram calibration curve demonstrates the high accuracy of nomograms constructed in this study (Figure 9B).

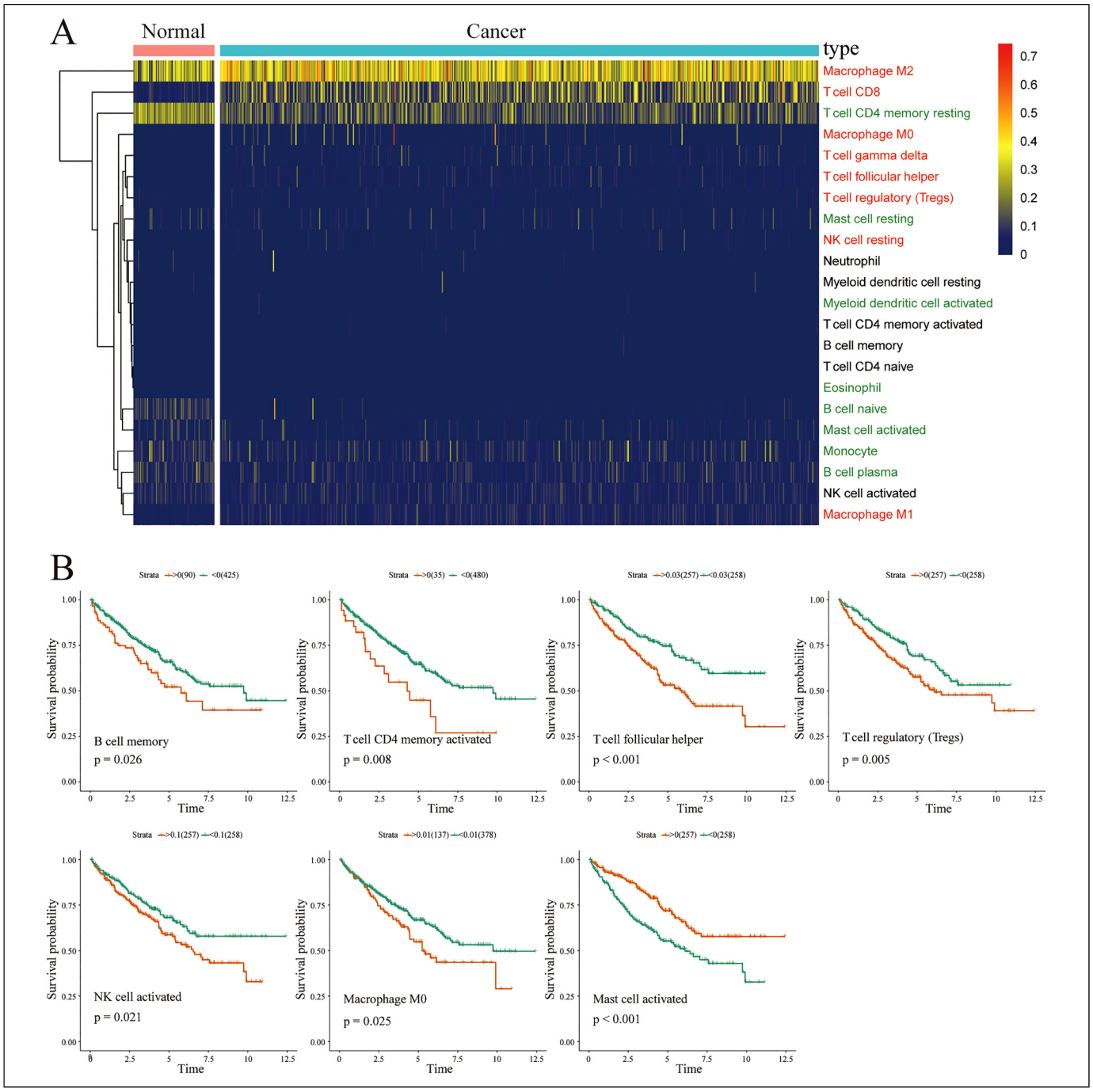

Figure 7. Analysis of immune cell infiltration in clear cell renal cell carcinoma (ccRCC) patients. (A) The heatmap shows the difference in immune cell infiltration between tumor and normal tissues. The red font represents the increased level of immune cell infiltration in tumor tissue; the green font represents the decreased level of immune cell infiltration in the tumor tissue; the black font represents no difference in the level of immune cell infiltration. (B) The prognostic value of immune cells in ccRCC patients.

Discussion

The tumor immune microenvironment is a complex ecosystem that has a crucial function in cancer progression and response to immunotherapy. Metastatic RCC patients are typically treated with immunotherapy-based regimens, showing promising efficacy and OS benefits.[20,21] The interaction mechanism between tumor cells and tumor microenvironment gives a novel research idea for the occurrence, metastasis and recurrence of ccRCC.[21] However, immune checkpoint blockade (ICB) therapy, such as PD-1 inhibitors, few patients are permitted to benefit from the actual clinical applications., and the criteria for predicting responsiveness are still limited.[22] Therefore, providing potential and novel biomarkers for prognostic

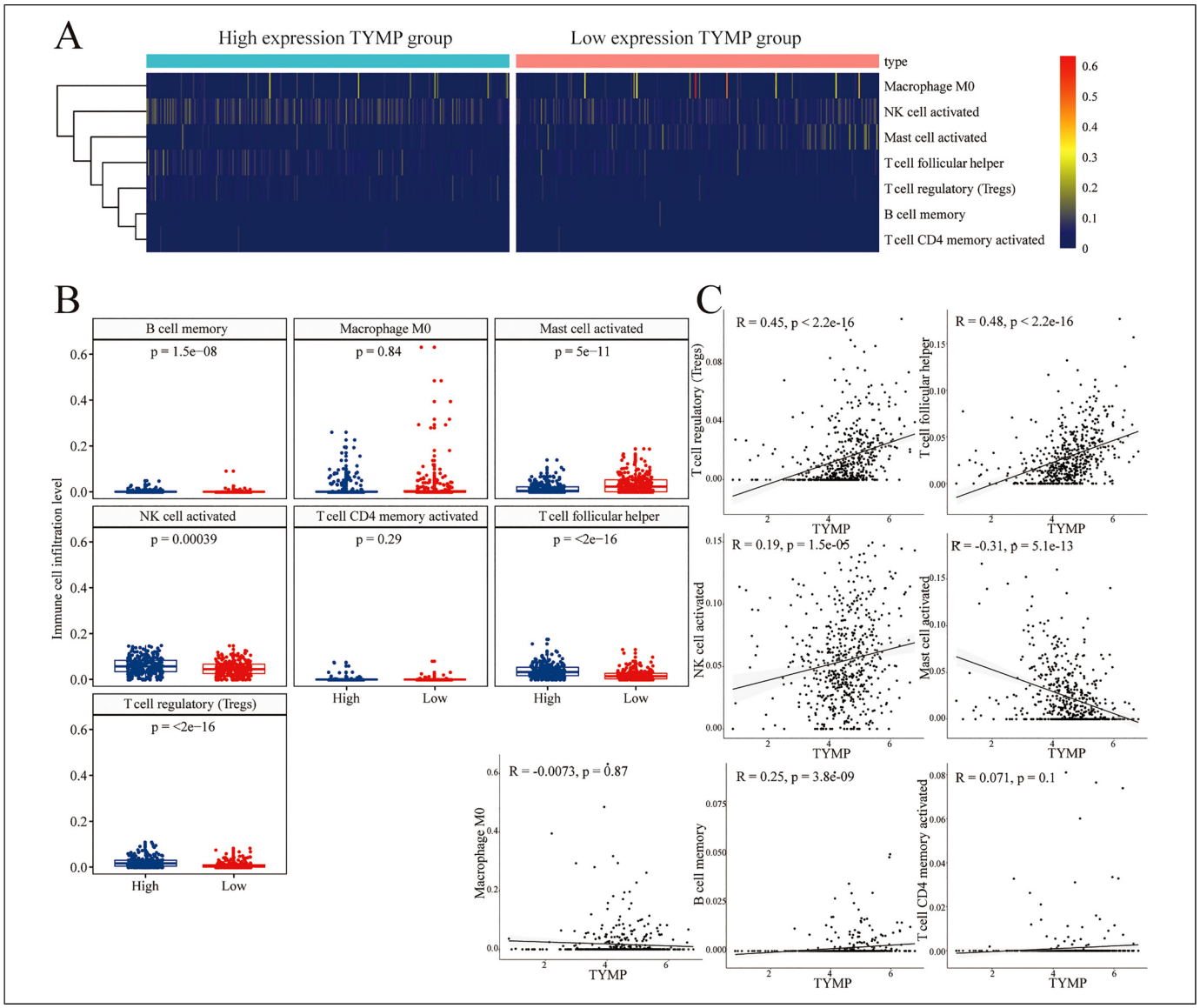

Figure 8. Infiltration levels in prognosis-related immune cells in the 2 thymidine phosphorylase (TYMP) expression groups. (A) A heatmap depicting the variation in immune cell infiltration between individuals with the 2 TYMP expression groups in relation to prognosis. (B) A box plot depicting the variation in immune cell infiltration levels between groups with high and low TYMP expression. (C) Correlation research revealed a link between TYMP and the level of immune cell infiltration linked to prognosis.

stratification of ccRCC and responsiveness to ICB therapy has become the current research interest.

TYMP, also known as TYMP, is found on chromosome 22q13.32-qter[23] and consists of ten exons that span approximately 4.3 kb. TYMP mRNA is 1.8 kb in length and encodes a 482-residue protein with an approximate 50 kDa molecular weight.[11,24] According to studies, breast and colorectal cancers are just 2 of the many solid tumors in which TYMP is overexpressed, and its primary method of action is to increase angiogenesis and anti-apoptosis.[25,26] Furthermore, investigations have shown that TYMP is substantially expressed in numerous types of solid malignancies, such as bladder, clear cell renal cell, breast, and esophageal cancers, lung, rectal, and gastric adenocarcinoma, and LUSC. A low expression was detected in pancreatic cancer. Differential expression analysis shows that high levels of TYMP expression in tumor tissues are linked to a worse prognosis in both clear cell renal carcinoma and LUSC. According to the results of a bioinformatics analysis, TYMP mRNA expression levels and protein were both elevated in ccRCC, and its expression level was linked to the clinical stage: the more advanced the tumor stage, the higher TYMP expression level. We confirmed that the TYMP expression level was elevated in renal carcinoma after collecting renal carcinoma specimens.

TYMP is a member of the pyrimidine nucleoside phosphorylase (PyNP) family, and its primary purpose is to move pyrimidine nucleosides through the salvage pathway.[27,28] Mechanistic studies have revealed that TYMP can directly

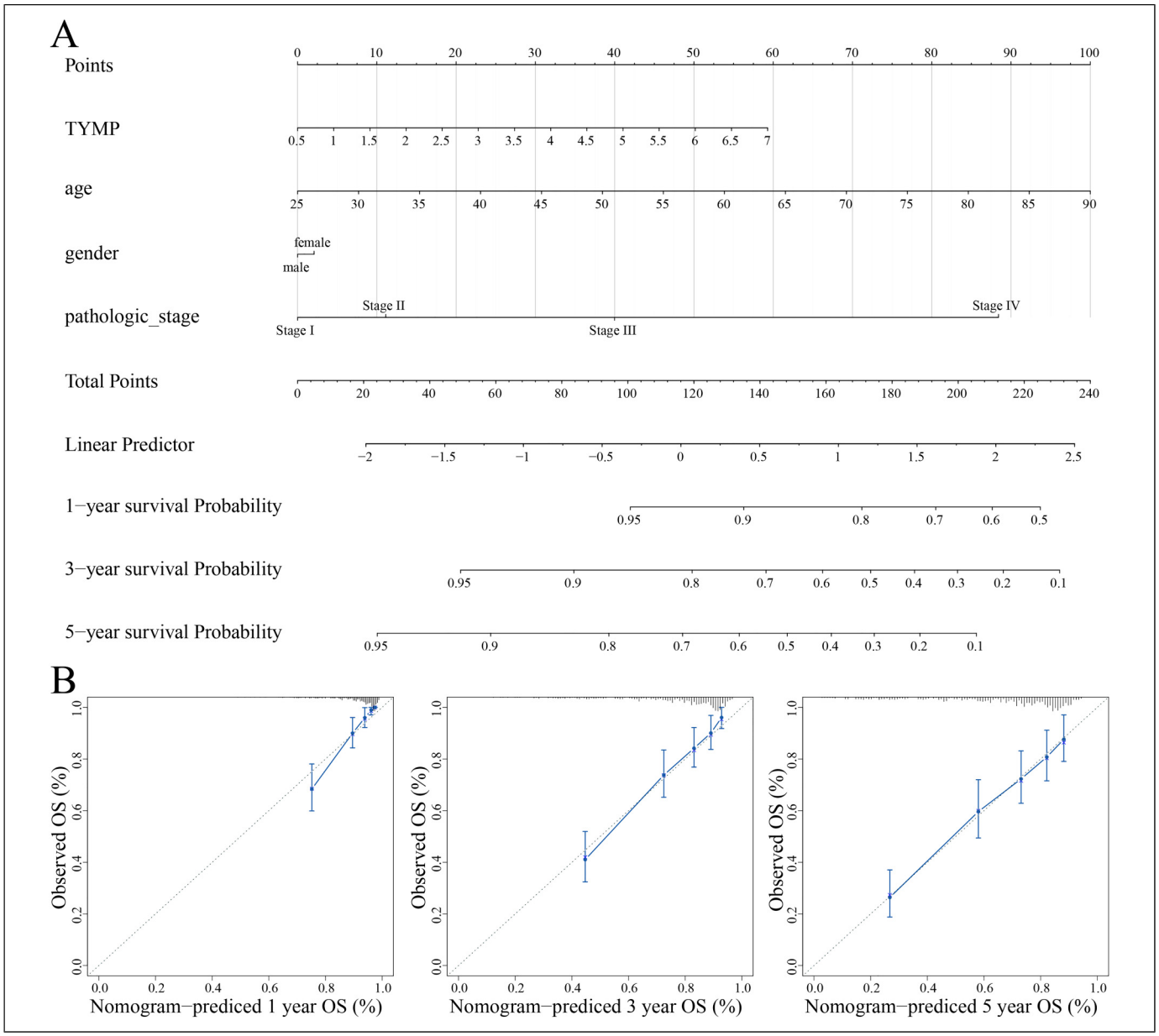

Figure 9. A prognostic model developed using thymidine phosphorylase (TYMP) expression levels and clinical characteristics. (A) The nomogram was developed using the expression level of TYMP and clinical characteristics. (B) The nomogram's internal validity is demonstrated by the calibration plot. Survival rate as observed (*Y* axis) and expected survival rate (*X* axis) are plotted using a nomogram.

bind to Src family kinase (SFK) through its N-terminal residue as a signaling molecule.[29] In addition, TYMP may affect the activities of focal adhesion kinase and integrins, which are related to its enzymatic activities.[30] The involvement of TYMP in controlling the aggressiveness of renal cancer cells was verified by functional studies conducted in vitro. To further study the probable mechanism of TYMP in clear cell renal cancer, we separated ccRCC patients into groups with high and low median TYMP expression levels. Differential genes identified based on the screening of the 2 groups include numerous immune-related genes, such as PDCD1, LAG3, CXCL9, CXCL13, and CCL5. Differentially expressed genes are predominantly involved in cytokine-cytokine receptor interaction, PD-L1 expression and PD-1 checkpoint pathway in cancer, viral protein interaction with cytokine and cytokine receptor, and T-cell receptor signaling pathway, according to KEGG analysis. GESA analysis also showed that TYMP may participate in immune regulation, fatty acid metabolism, and the P53 signaling pathway.

We examined the differentially invading immune cells linked to the prognosis of ccRCC to further investigate the role of TYMP in immune regulation. A dataset of immune cell infiltration levels in the TCGA-KIRC cohort was also obtained for differential and prognostic studies. In ccRCC,

patients with elevated levels of invasive T-cell follicular helper, T-cell regulatory (Tregs), and Macrophage M0 had lower outcomes. TYMP expression level in ccRCC was significantly connected with T-cell follicular helper and Tregs, but negatively correlated with mast cell activation. The infiltration level of T-cell follicular helper and Tregs was higher in the high TYMP expression group compared to the low TYMP expression group. In contrast, the level of infiltration in the mast cell activation was lower. Therefore, we preliminarily believe that TYMP may regulate tumor-associated immune cell infiltration through T-cell follicular helper, Tregs, and activated mast cells, therefore impacting the onset and progression of renal cancer with clear cell differentiation.

This study also has some limitations. Previous studies have confirmed that TYMP can regulate the proliferation, apoptosis, and migration, but its molecular mechanism has not been thoroughly studied. Through bioinformatics analysis, it was found that TYMP can regulate immune cell infiltration, but we did not further explain its regulatory mechanism.

Conclusion

TYMP is substantially expressed in multiple cancers, consisting of ccRCC, and its high expression level is significantly connected with clear cell renal carcinoma's worse prognosis. The findings indicate the significance of TYMP in controlling the aggressiveness of renal cancer cells. TYMP may also affect the evolution of ccRCC by modulating the infiltration level of tumor-related immune cells, such as T-cell follicular helper, Tregs, and activated mast cells. On the basis of TYMP expression levels and the clinical features of patients, we developed a predictive nomogram for ccRCC patients that reliably predicted OS. The data imply that TYMP is a potential immune-related biomarker and clinically significant target in patients with ccRCC.

Author Contributions

SC performed the experiments and revised the manuscript. JC designed the study and collected the data. JZ wrote the manuscript, collected the data, performed data analysis, interpretation, and revised the final manuscript. NW collected the data and performed data analysis. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

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Ethical Approval

The studies involving human participants were reviewed and approved by The Ethics Committee of Shandong Provincial Hospital Affiliated with Shandong First Medical University, and the approval number was NO.SWYX2023-239.

Informed Consent

The patients/participants provided their written informed consent to participate in this study.

Data Availability

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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Supplemental Material

Supplemental material for this article is available online.

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