

Identification and Validation of a Prognostic Signature Based on mRNAs Associated with Low-Density Lipoprotein Receptor-Related Proteins for Kidney Renal Clear Cell Carcinoma: Insights Into Tumor Immune Microenvironment, Mutation Patterns, and Personalized Treatment Strategies

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Background: Low-density lipoprotein receptor-related protein (LRP) is integral to protein synthesis and contributes significantly to tumor initiation and growth. However, the role of LRP-related mRNAs (LRPMRs) in KIRC progression remains unclear. Our study investigates the potential use of LRPMRs as prognostic markers in patients with KIRC.

Methods: Clinical and transcriptomic data of KIRC patients were obtained from The Cancer Genome Atlas (TCGA) database for model construction and performance evaluation. A nomogram integrating clinical characteristics and the risk model was then established. To explore the clinical significance and underlying mechanisms, we analyzed the tumor microenvironment (TME), evaluated tumor mutational burden (TMB), performed gene set enrichment analysis, and predicted drug sensitivity. The mRNA expression was assessed using RT-qPCR.

Results: A six-LRPMR-based model was developed and provided significant prognostic information. Kaplan-Meier analysis demonstrated worse survival outcomes for high-risk (H-R) patients ($p < 0.001$). A nomogram incorporating the risk model showed improved predictive accuracy compared with the clinical model alone ($AUC = 0.761$). GSEA highlighted proximal tubule transport and propanoate metabolism pathways as significantly enriched in the low-risk (L-R) group, while the H-R group displayed enrichment in CD22-mediated BCR regulation and FCGR activation pathways. Higher TMB in the H-R cohort predicted a poor prognosis. TME analysis suggested that H-R patients may respond less favorably to immunotherapy. Drug sensitivity analysis indicated that H-R patients were more sensitive to Staurosporine and Sabutoclax, whereas L-R patients were more sensitive to dihydrorotenone and osimertinib. RT-qPCR validated differential mRNA expression between KIRC and normal cells.

Conclusion: This six-LRPMR-based prognostic model provides valuable insights for prognosis assessment and personalized treatment selection in KIRC.

Keywords: low-density lipoprotein receptor-related protein, mRNAs, kidney renal clear cell carcinoma, prognostic model, nomogram

Introduction

The renal clear cell carcinoma of kidney (KIRC) constitutes 75–80% of all kidney cancer diagnoses, making it the predominant subtype of renal cell carcinoma.¹ Despite improvements in diagnostic and therapeutic approaches, the

prognosis for patients with KIRC continues to be unfavorable, particularly in advanced or metastatic stages.² The molecular mechanisms driving KIRC and its tumor microenvironment (TME) remain incompletely understood.³ To address the limitations of the American Joint Committee on Cancer (AJCC) Tumor-Node-Metastasis (TNM) staging system in prediction accuracy, alternative methods are needed for more precise prognosis assessment.^{4,5} Recently, significant potential has been demonstrated by biomarker-based models in cancer prognosis.

Low-density lipoprotein receptor-related proteins (LRPs) are multifunctional receptors involved in endocytosis, lipid metabolism, and signal transduction. Dysregulation of LRP signaling has been linked to tumor invasion, angiogenesis, and immune modulation in KIRC.⁶ Importantly, LRPs interact with specific mRNAs that mediate downstream oncogenic processes, including extracellular matrix remodeling and immune evasion.⁷ Therefore, exploring LRP-related mRNAs provides a biologically grounded approach for identifying prognostic biomarkers in KIRC.⁸ LRP has been implicated in the progression of various cancers by modulating key processes such as cell proliferation, apoptosis, and interactions within the TME.⁹ Recent research has emphasized the impact of messenger RNA (mRNA) on the progression of KIRC, particularly through the modulation of tumor cell signaling pathways.¹⁰ Given these findings, LRP has emerged as a promising therapeutic target, particularly for modulating the metabolic and growth pathways of tumor cells.¹¹ In addition, prognostic models based on mRNA expression, such as those developed by Tu et al for peripheral T-cell lymphoma and Li et al for cervical carcinoma, have shown great potential in predicting patient outcomes.^{12,13} Consequently, we developed an LRP-related mRNAs (LRPMRs) model to explore its potential as a novel therapeutic strategy for KIRC patients.

In our study, we performed a detailed bioinformatics analysis of LRPMRs. By combining expression data and clinical data of KIRC patients, we developed a prognostic model and conducted prognostic prediction and mechanistic exploration to investigate its potential role in tumor progression.¹⁴ Our study not only reveals potential associations between LRP and KIRC but also provides new insights for future targeted therapies.

Materials and Methods

Sources of Data and Accessibility

The data for our study came from the UCSC Xena platform (<https://xena.ucsc.edu/>, on August 27, 2024). Specifically, transcriptome data from 607 KIRC patients and clinicopathologic details from 979 cases were acquired from The Cancer Genome Atlas (TCGA). The transcriptome dataset includes HTSeq count and HTSeq FPKM format, supporting a variety of analysis methods. In addition, we extracted somatic mutation data for tumors via TCGA site (<https://portal.gdc.cancer.gov/repository>, on August 27, 2024). Only patients with comprehensive transcriptional and clinical data were included, with a final cohort of 597 samples. We obtained the E-MTAB-1980 cohort (E-MC) from the ArrayExpress repository (<https://www.ebi.ac.uk/>) and used it for external validation.¹⁵ To minimize potential batch effects between TCGA and E-MTAB-1980 datasets, we applied the “ComBat” function in the sva R package before model training and validation.

Identification of LRP-Associated Genes and LRPMRs

To identify LRP-associated genes, a search for LRP was performed using the data from GeneCards (<https://www.genecards.org/>, on August 27, 2024). The relationship between LRP-related genes and all mRNAs was evaluated using Pearson correlation analysis, with LRPMRs determined based on both relevance coefficient > 0.3 and $p < 0.05$. Differential expression of mRNAs was evaluated using the “DESeq2” package, with thresholds of $|\log_2\text{FoldChange}| > 1$ and $p < 0.05$. Patients were randomly split (1:1) into train and test cohorts.¹⁶ Finally, we performed univariate Cox regression analysis (uCoxRA), Least Absolute Shrinkage and Selection Operator (LASSO) regression analysis, and multivariate Cox regression analysis (mCoxRA) to obtain the final LRPMR.

Development and Validation of the Prognostic Model

We utilized the findings to construct a predictive model, where the risk score was calculated using the following formula: risk score = (coefficient of mRNA1 × expression level of mRNA1) + (coefficient of mRNA2 × expression level of

mRNA2) + (coefficient of mRNA3 × expression level of mRNA3) + ... + (coefficient of mRNA_n × expression level of mRNA_n). Patients in the train, test, and overall cohorts were grouped into low-risk (L-R) and high-risk (H-R) categories according to the midpoint of the risk score. We performed Kaplan-Meier (K-M) analyses using the “survminer” and “survival” R packages to evaluate the survival disparities between the H-R and L-R groups and the overall survival (OS) of patients. Receiver operating characteristic (ROC) curves were generated to assess the predictive performance of the model at 1, 3, and 5 years.

In order to assess the efficacy of the model, we performed principal component analysis (PCA) on the gene expression profiles, incorporating LRPMRs, mRNAs, and the genes related to the prognostic framework.¹⁷ Furthermore, uCoxRA and mCoxRA that included risk scores and clinical variables were conducted to confirm the model’s prognostic value. K-M survival analysis was conducted to investigate the relationship between the prognostic tool and clinical factors.

Creation of Nomogram to Predict Patient Survival

We created a nomogram using the “RMS” package, integrating age, stage, and risk group to predict OS over the course of 1, 3, and 5 years. Calibration and ROC curves assessed its predictive utility.¹⁸

Enrichment Analysis (EA)

KEGG EA was conducted to investigate the mechanisms and functions potentially associated with specific gene sets. Based on the simulation framework involving six LRPMRs, participants were categorized into H-R and L-R groups. EA was subsequently conducted using the “org.Hs.eg.db” “enrichplot” and “clusterProfiler” R packages to identify pathways with false discovery rates under 0.25 and $p < 0.05$.¹⁹

Tumor Mutational Burden (TMB)

Data on tumor somatic mutations were sourced from the TCGA database and processed through the “TCGA biolinks” package. Waterfall plots were generated with the “maftools” package in R, and TMB was calculated.²⁰

Tme

The R package “ESTIMATE” was applied to evaluate the scores with variations in stromal, immune, ESTIMATE, and tumor purity between the H-R and L-R group. We acquired supplementary data from the Tumor Immune Estimation Resource (TIMER) 2.0 site (<http://timer.cistrome.org/>, until August 27, 2024). We employed seven computational tools—XCELL, CIBERSORT, CIBERSORT-ABS, QUANTISEQ, TIMER, EPIC, and MCPCOUNTER—to enhance the analysis of associations between immune cell types and risk scores.²¹ In addition, an assessment of immune cell infiltration was conducted across 22 immune cell subtypes to further examine shifts in immune cell composition.

Immunotherapy Vs Chemotherapy

To assess immune dysfunction in KIRC patients, we retrieved Tumor Immune Dysfunction and Exclusion (TIDE) scores and data from the TIDE platform (<http://tide.dfci.harvard.edu/>, accessed on August 27, 2024) and performed single-sample gene set enrichment analysis (ssGSEA) using the “GSVA” R package.²² The half-maximal inhibitory concentration (IC50) values of common chemotherapy drugs were calculated with the “oncoPredict” package in R and compared across groups using the Wilcoxon signed-rank test.

RT-qPCR and HPA

The normal renal proximal tubular epithelial cell line (HK-2) and renal carcinoma cell lines (786-O, A-498 and ACHN) were obtained from Procell life science and technology co. ltd. We isolated RNA using TRIzol Reagent (Life Technologies, CA, USA), synthesized cDNA using the PrimeScript RT Kit (Takara, Japan). Gene expression was then analyzed through RT-qPCR.²³ The primers for LRPMRs are listed in [Table S1](#). The mRNA expression levels were quantified using the $2^{-\Delta\Delta Ct}$ method.

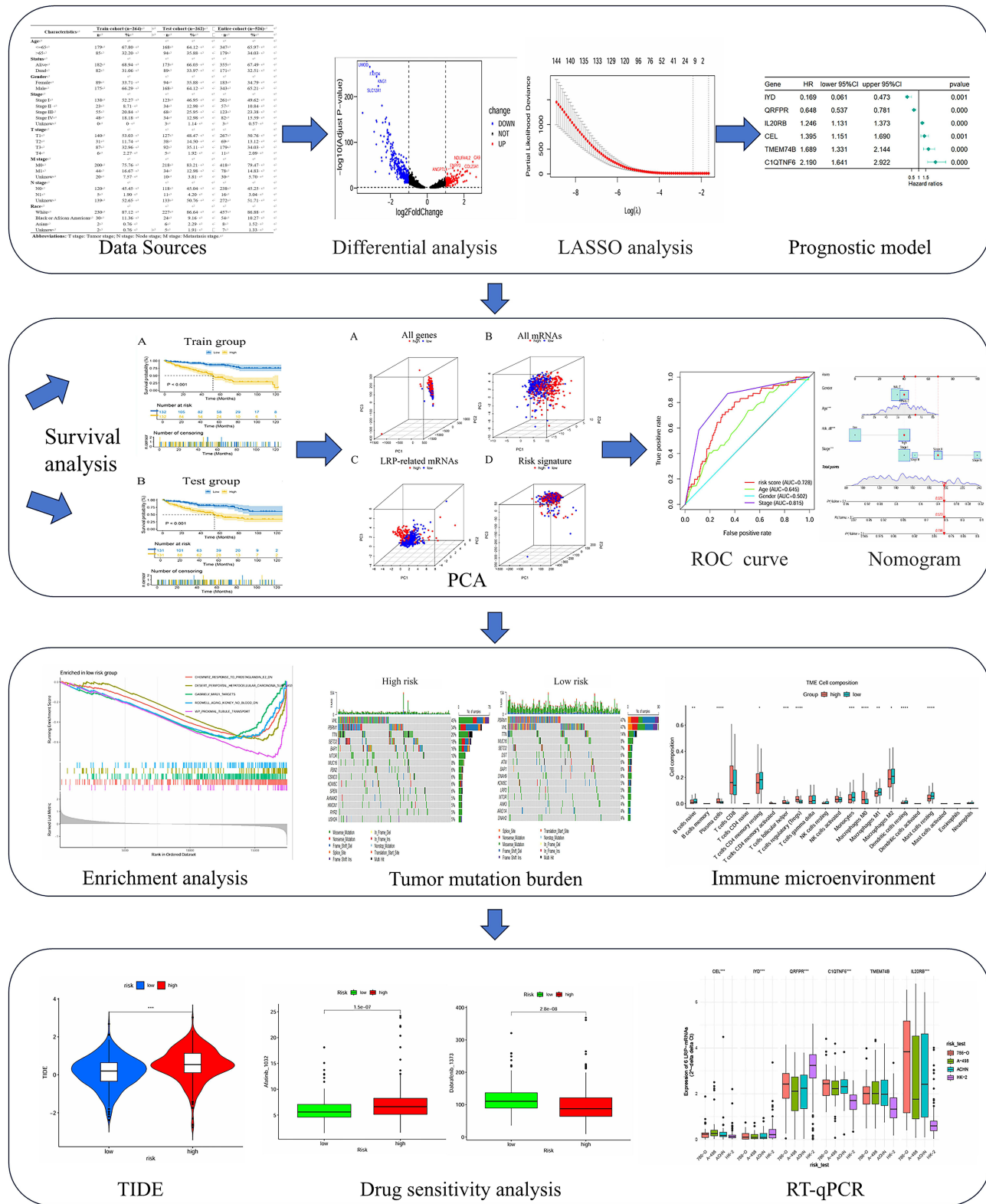


Figure 1 Flow Chart. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

To assess the expression of LRP-related proteins in KIRC versus normal tissues, protein expression levels were analyzed in this study using resources available on the Human Protein Atlas (HPA) platform (<https://www.proteinatlas.org/>).

Construct an External Validation of LRPMRs

We used the E-Mc cohort for external validation to assess the applicability of the LRPMR-based model. Initially, we analyzed the relationship between risk scores and clinical features (age, sex, stage, and risk score) using the chi-square test in the E-Mc cohorts. Survival analyses are then performed to predict patient outcomes. Independent prognostic factors were identified through u/mCoxRA. To estimate OS at 1, 3, and 5 years for KIRC, we utilized the “rms”, “timeROC”, and “tidyverse” R packages, ensuring robust survival predictions.

Results

Identification of LRPMRs in KIRC Patients

A flowchart (Figure 1) outlined the strategy of our research. LRP-related genes were first identified, and a protein-protein interaction (PPI) network was constructed for 36 of these genes through the STRING platform (Figure 2A). In the following step, Pearson correlation analysis identified 14,000 LRPMRs, which were subsequently examined for differential expression to determine those significantly altered in tumor tissues (Figure 2B).

Based on these results, a total of 526 patients were randomly split between the train and test cohorts (Table 1). The uCoxRA was utilized in the train cohort to identify 157 prognostic mRNAs, with a significance threshold of $p < 0.05$ (Table S2). Further LASSO analysis narrowed these down to nine differentially expressed LRPMRs (Figure 2C, D and Table S3), and mCoxRA identified the six most significant mRNAs (Figure S1A). The relationships among these six mRNAs were further analyzed (Figure S1B), including their correlation with LRP-related genes (Figure S1C). Notably, these mRNAs exhibited significant differential regulation in tumor tissues, with clear patterns of up- and down-regulation (Figure S1D).

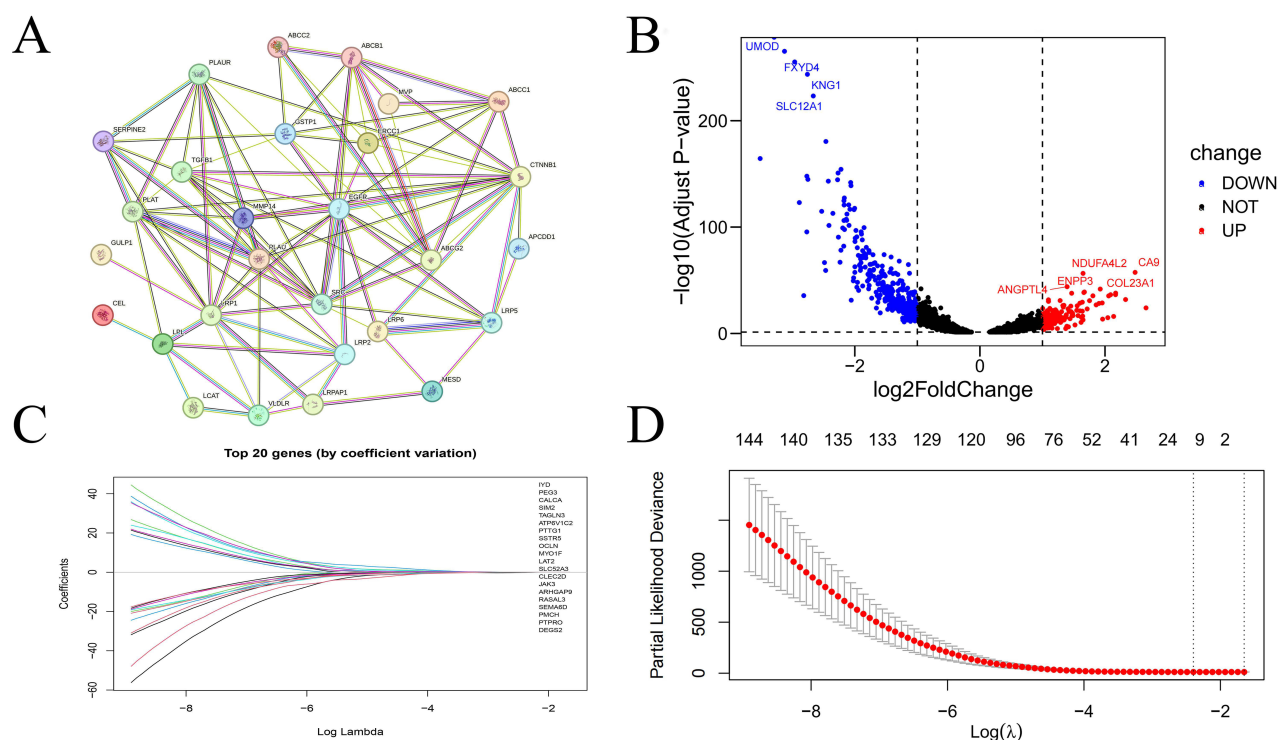


Figure 2 Filter for LRPMRs. PPI network among 36 LRP-related genes (A); Volcano plot of differentially expressed LRP-associated mRNAs (B); LASSO regression analysis (C and D).

Table 1 Clinical Information of the Patients in the Test and Train Groups

Characteristics	Train Cohort (n=264)		Test Cohort (n=262)		Entire Cohort (n=526)	
	n	%	n	%	n	%
Age						
<=65	179	67.80	168	64.12	347	65.97
>65	85	32.20	94	35.88	179	34.03
Status						
Alive	182	68.94	173	66.03	355	67.49
Dead	82	31.06	89	33.97	171	32.51
Gender						
Female	89	33.71	94	35.88	183	34.79
Male	175	66.29	168	64.12	343	65.21
Stage						
Stage I	138	52.27	123	46.95	261	49.62
Stage II	23	8.71	34	12.98	57	10.84
Stage III	55	20.84	68	25.95	123	23.38
Stage IV	48	18.18	34	12.98	82	15.59
Unknown	0	0	3	1.14	3	0.57
T stage						
T1	140	53.03	127	48.47	267	50.76
T2	31	11.74	38	14.50	69	13.12
T3	87	32.96	92	35.11	179	34.03
T4	6	2.27	5	1.92	11	2.09
M stage						
M0	200	75.76	218	83.21	418	79.47
M1	44	16.67	34	12.98	78	14.83
Unknown	20	7.57	10	3.81	30	5.70
N stage						
N0	120	45.45	118	45.04	238	45.25
N1	5	1.90	11	4.20	16	3.04
Unknown	139	52.65	133	50.76	272	51.71
Race						
White	230	87.12	227	86.64	457	86.88
Black or African American	30	11.36	24	9.16	54	10.27
Asian	2	0.76	6	2.29	8	1.52
Unknown	2	0.76	5	1.91	7	1.33

Abbreviations: T stage, Tumor stage; N stage, Node stage; M stage, Metastasis stage.

Establishing and Verification the Prognostic Model

Using the six LRPMRs, we used a formula to calculate risk scores for each patient: Risk score = coefficient (CEL) × expression (CEL) + coefficient (IYD) × expression (IYD) + coefficient (QRFPR) × expression (QRFPR) + coefficient (IL20RB) × expression (IL20RB) + coefficient (TMEM74B) × expression (TMEM74B) + coefficient (C1QTNF6) × expression (C1QTNF6). Clinical characteristics of patients in the H-R and L-R groups are summarized in Table 2. Survival analysis demonstrated notably poorer outcomes for H-R patients compared to L-R patients across the train, test, and entire cohorts (all $p < 0.001$, Figure 3A–C). We further confirmed the robust predictive capacity through Time-dependent ROC curve analysis (TdROC), which yielded the following AUC values: Train cohort: 1 year: 79.56%, 3 years: 80.44%, 5 years: 82.08%; Test cohort: 1 year: 69.16%, 3 years: 66.45%, 5 years: 71.24%; Total cohort: 1 year: 73.18%, 3 years: 72.32%, 5 years: 76.08% (Figure 3D–F).

Additional analyses, including the expression profiles of the six mRNAs in different risk groups (Figure S2A–C), risk curves (Figure S2D–F), risk distribution plots (Figure S2G–I), and scatter plots (Figure S2J–L), confirmed the predictive accuracy of the model. PCA results (Figure 4A–D) revealed significant aggregation in the H-R and L-R groups, respectively.

Table 2 Clinical Information for 526 Patients in Different Risk Categories

Characteristics	High-Risk Group (n=263)		Low-Risk Group (n=263)	
	n	%	n	%
Age				
≤65	167	63.50	180	68.44
>65	96	36.50	83	31.56
Status				
Alive	134	50.95	221	84.03
Dead	129	49.05	42	15.97
Gender				
Female	84	31.94	93	35.36
Male	179	68.06	170	64.64
Stage				
Stage I	100	38.02	161	61.22
Stage II	26	9.89	31	11.79
Stage III	74	28.14	49	18.63
Stage IV	60	22.81	22	8.36
Unknown	3	1.14	0	0
T stage				
T1	105	39.92	162	61.60
T2	32	12.17	37	14.07
T3	116	44.11	63	23.95
T4	10	3.80	1	0.38
M stage				
M0	187	71.10	231	87.83
M1	57	21.67	21	7.98
Unknown	19	7.23	11	4.19
N stage				
N0	117	44.49	121	46.01
N1	14	5.32	2	0.76
Unknown	132	50.19	140	53.23
Race				
White	231	87.83	226	85.93
Black or African American	28	10.65	26	9.89
Asian	2	0.76	6	2.28
Unknown	2	0.76	5	1.90

Abbreviations: T stage, Tumor stage; N stage, Node stage; M stage, metastasis stage.

In uCoxRA, age (HR for 1.03, 95% CI amount 1.02–1.05, p less than 0.001), stage (HR for 1.95, 95% CI amount 1.62–2.35, p less than 0.001) and risk score (HR for 1.57, 95% CI amount 1.40–1.77, p less than 0.001) emerged as significant independent predictors of prognosis (Figure 4E). These findings were confirmed by mCoxRA, where age (HR for 1.04, 95% CI amount 1.02–1.06, p less than 0.001), stage (HR for 1.88, 95% CI amount 1.54–2.30, p less than 0.001), and risk score (HR for 1.36, 95% CI amount 1.20–1.55, p less than 0.001) were validated as key risk indicators (Figure 4F).

Figure S3A presents an expression heatmap that combines clinical data, risk categories, and mRNAs associated with the model, underscoring its predictive value. Survival differences based on age (≤ 65 : $p < 0.001$; > 65 : $p < 0.001$), sex (male: $p < 0.001$; female: $p < 0.001$), stage (I–II: $p < 0.001$; III–IV: $p < 0.001$) are further depicted in the survival curves shown in Figure 5.

Nomogram for Predicting Clinical OS

The scatter plot (Figure S3C) revealed a significant positive correlation of stage with risk scores ($p < 0.001$). The scatter plot (Figure S3B) showed that age was not associated with risk score ($p > 0.05$). Both ROC curve analysis (Figure 6B) and DCA

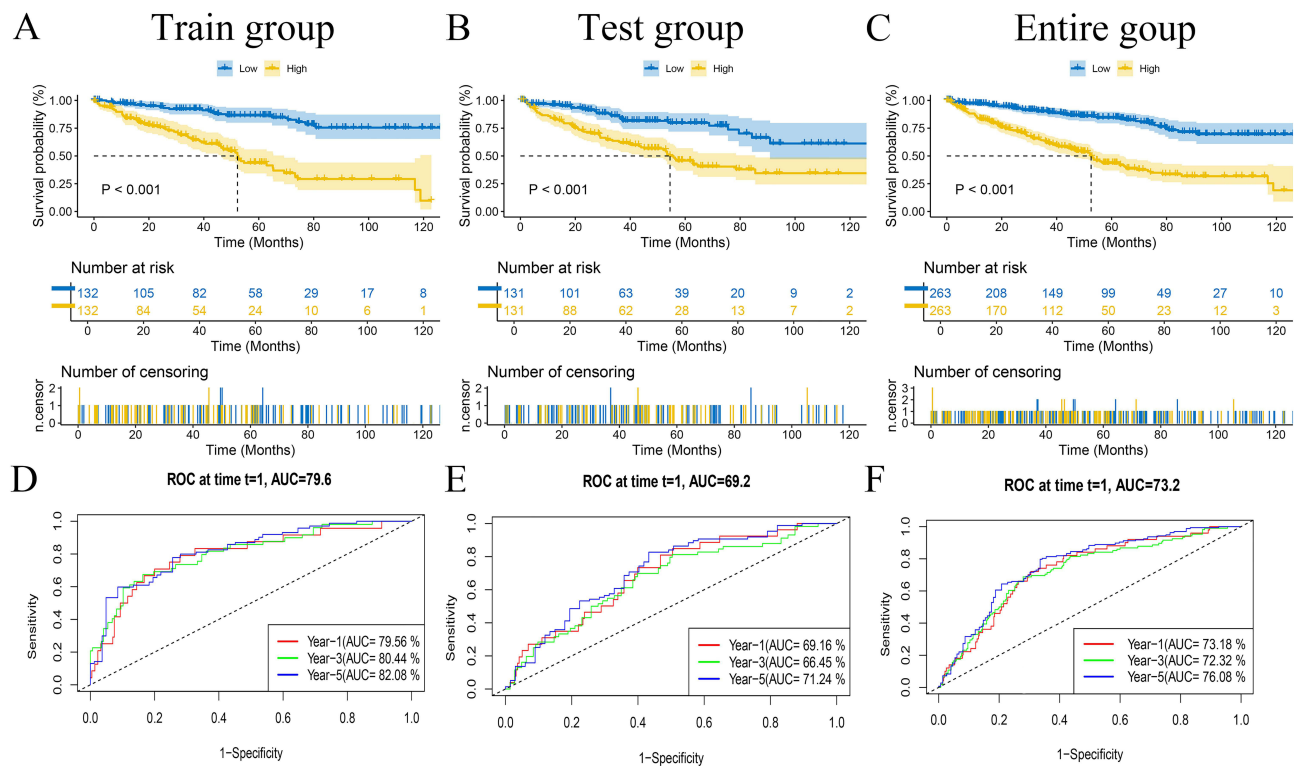


Figure 3 The model prediction effect is validated by the train group, test group, and entire group. K-M analysis (A–C) and TdROC curves (D–F) to compare the survival of the H-R group and L-R group.

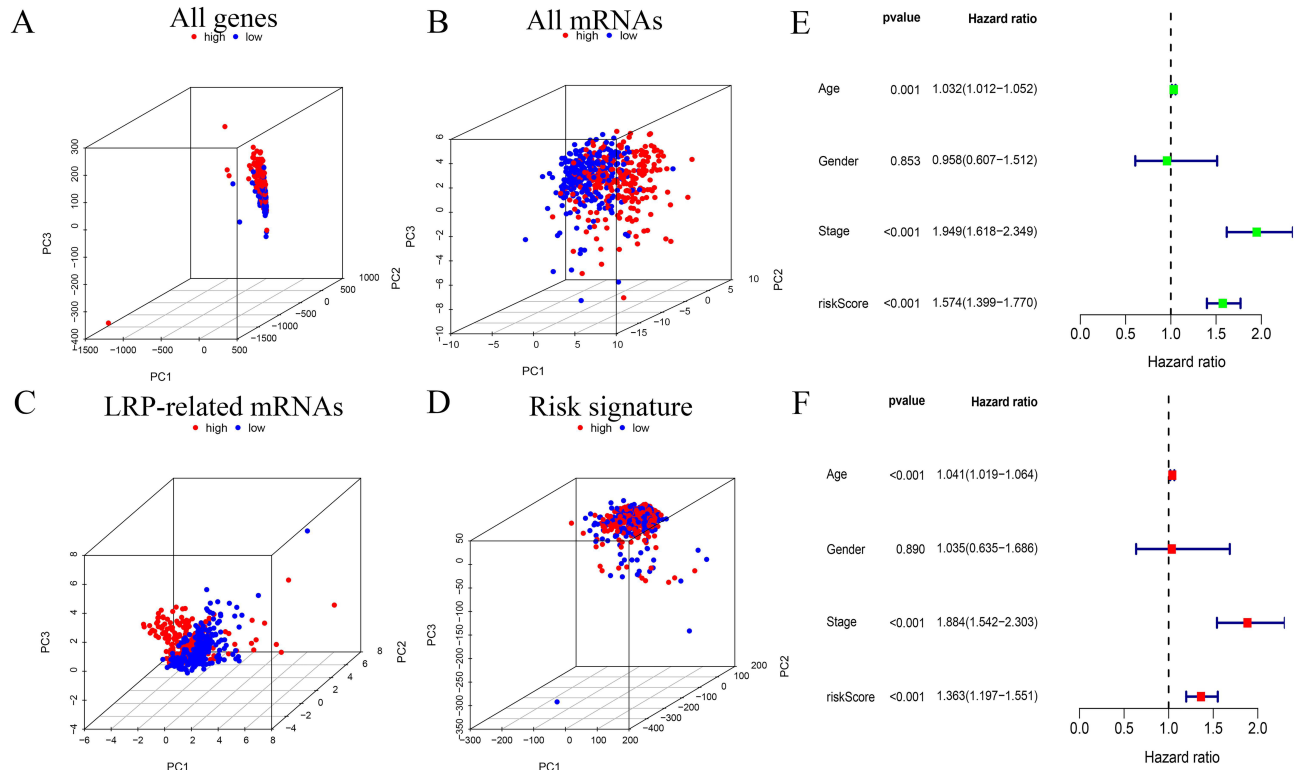


Figure 4 PCA and independent prognostic analysis of the signature. PCA based on all genes (A), all mRNAs (B), LRP-related mRNAs (C), and risk signature (D); Univariate (E) and multivariate (F) independent prognostic analysis.

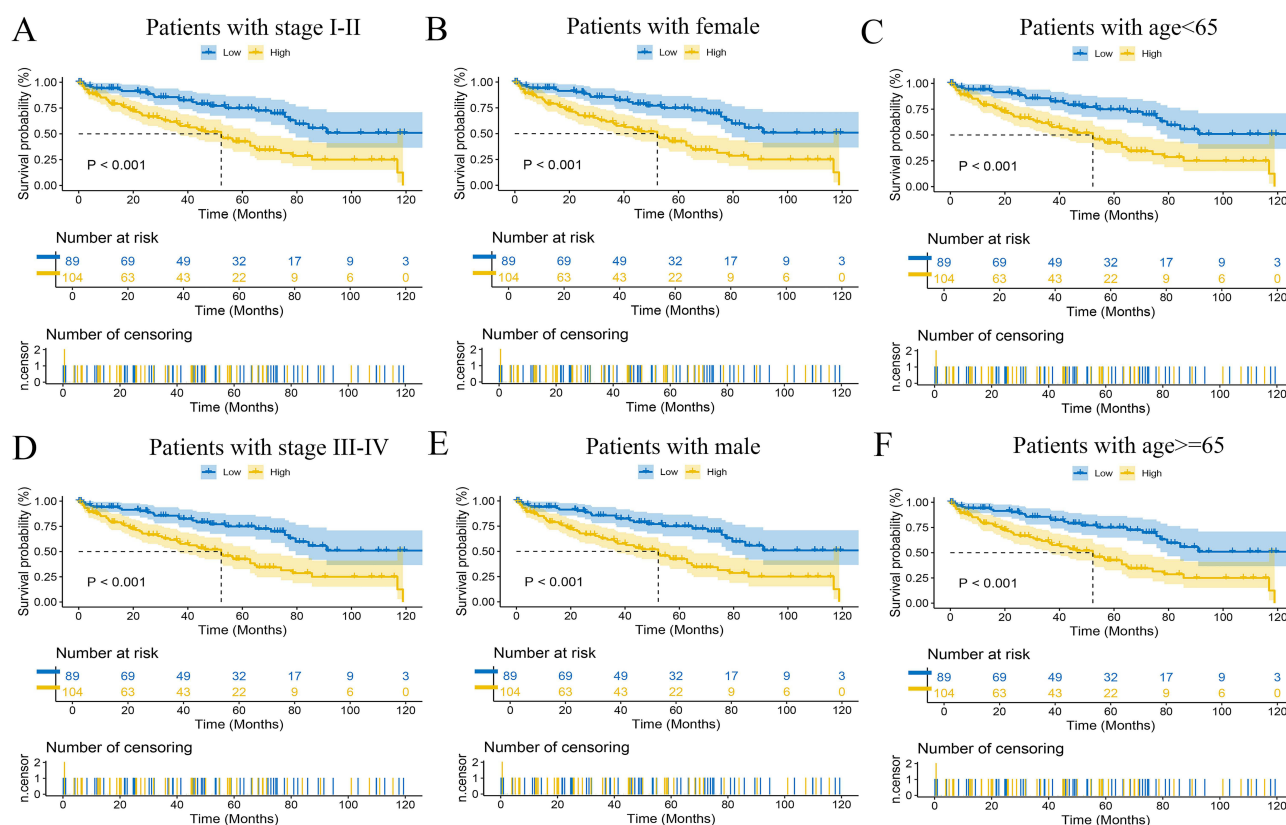


Figure 5 Further validation of model effects. Survival curves of patients in different clinical states (A–F).

(Figure 6A) showed that risk scores were more predictive of outcomes compared to other clinical factors, with AUC values of 0.728 for risk, 0.645 for age, 0.502 for sex, and 0.815 for stage. A prospective estimator incorporating age, risk, sex, and stage was developed for KIRC patients (Figure 6C) and validated with promising results (Figure S4). The nomogram using the risk model (Figure 6D) showed higher predictive accuracy compared to the model without it (Figure 6E).

Enrichment Analysis

KEGG EA identified key functions of differentially expressed genes, such as endocytosis, focal adhesion, axon guidance, and proteoglycans in cancer (Figure S5). GSEA revealed distinct pathways enriched in different gene sets. In reactor EA, the H-R gene set was linked to CD22-mediated BCR regulation and FCGR activation, while the L-R gene set was associated with propanoate metabolism and proximal tubule transport (Figure S6). All pathway details are provided in Table S4.

TMB

TMB levels were higher in the H-R cohort (Figure 7A). A subsequent waterfall plot illustrated the 15 most frequently mutated genes across different subgroups (Figure 7B and C). In H-R patients, the five most frequently variation genes were VHL (45%), PBRM1 (34%), TTN (20%), SETD2 (18%), and BAP1 (16%). In L-R patients, the most common mutations were in VHL (47%), PBRM1 (47%), TTN (14%), MUC16 (8%), and SETD2 (7%).

Analysis of TME

TME analysis revealed elevated immune (Figure 8A) and ESTIMATE scores (Figure 8C) ($p < 0.001$) in the H-R group, while stromal scores (Figure 8B) showed no significant variation. In addition, H-R patients showed a notable reduction in tumor purity ($p < 0.001$, Figure 8D), suggesting a tumor microenvironment that is immunosuppressive and could impair antitumor immunity.

Immune cell analysis using different computational methods (Figure 8E) revealed an increased abundance of M1 macrophages ($p < 0.01$), resting mast cells ($p < 0.0001$), follicular helper T cells ($p < 0.001$), Tregs ($p < 0.0001$), and M0

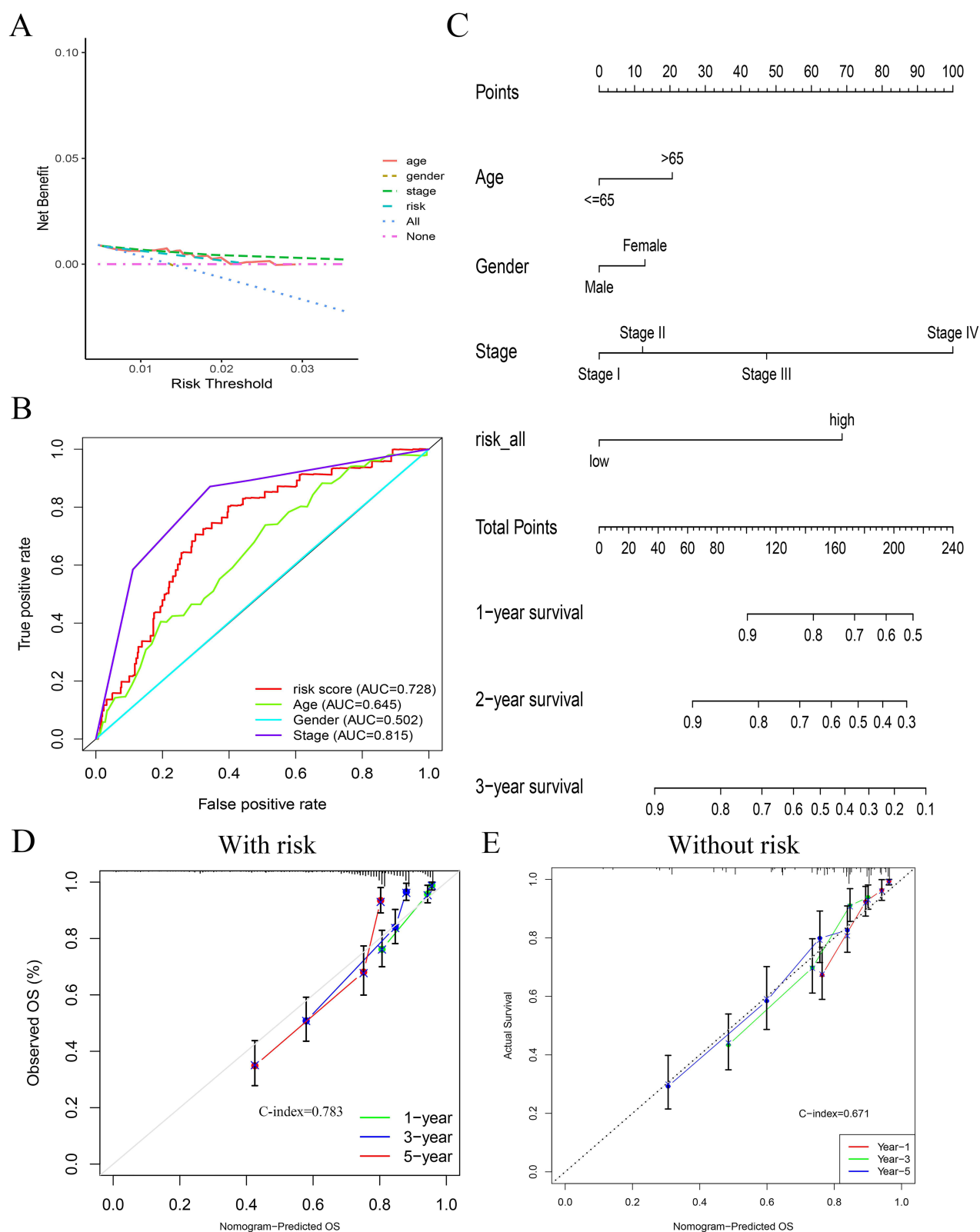


Figure 6 Nomogram predicts patient prognosis. Decision curve to test for forecast value (A); ROC curves containing different clinical information (B); A clinical prognosis nomogram is constructed by age, gender, risk, and stage together (C). Nomogram with (D) and without (E) risk model.

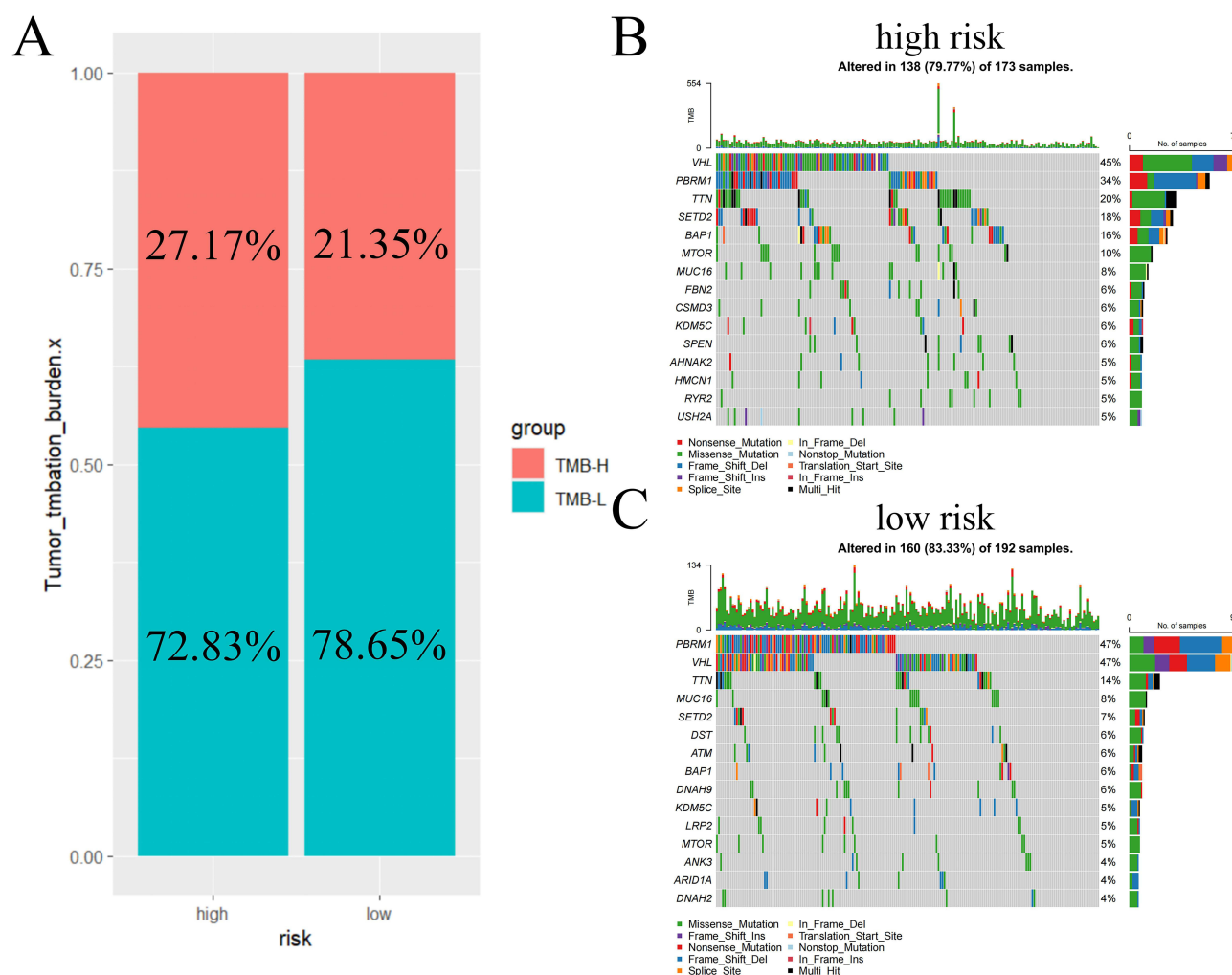


Figure 7 Tumor mutation burden in different risk groups. Percentage bar graph showing TMB for different risk subgroups (A); H-R group waterfall chart (B); L-R group waterfall chart (C).

macrophages ($p < 0.0001$) in the H-R group. On the contrary, resting memory CD4 T cells ($p < 0.05$), monocytes ($p < 0.001$), naive B cells ($p < 0.01$), and plasma cells ($p < 0.0001$) were more prevalent in L-R group (Figure 8F). Figure S7 illustrates the correlation between immune cells and risk scores.

Functional analysis revealed a reduction in type II IFN response ($p < 0.001$) and Antigen-presenting Cell (APC) coinhibition ($p < 0.001$) in H-R KIRC patients, whereas pathways such as parainflammation ($p < 0.01$) were upregulated (Figure 8G). These findings imply that tumor progression in H-R patients may be facilitated by parainflammatory responses and T cell costimulatory mechanisms.

Prediction of Treatment Outcomes in KIRC

TIDE scores showed increased levels of T cell exclusion and dysfunction in H-R patients with LRP-related signatures (Figure S8). These findings suggest that immune escape is more likely in the H-R subgroup, which could complicate treatment strategies. Drug sensitivity analysis revealed antitumor agents with diverse mechanisms. Drugs effective in L-R tumors are listed in Table S5, those more effective in H-R tumors are summarized in Table S6, and drugs with no significant sensitivity differences are shown in Table S7. Notably, H-R tumors showed enhanced sensitivity to treatments targeting the PI3K/mTOR and RTK pathways, providing important insights for personalized therapy and treatment planning.

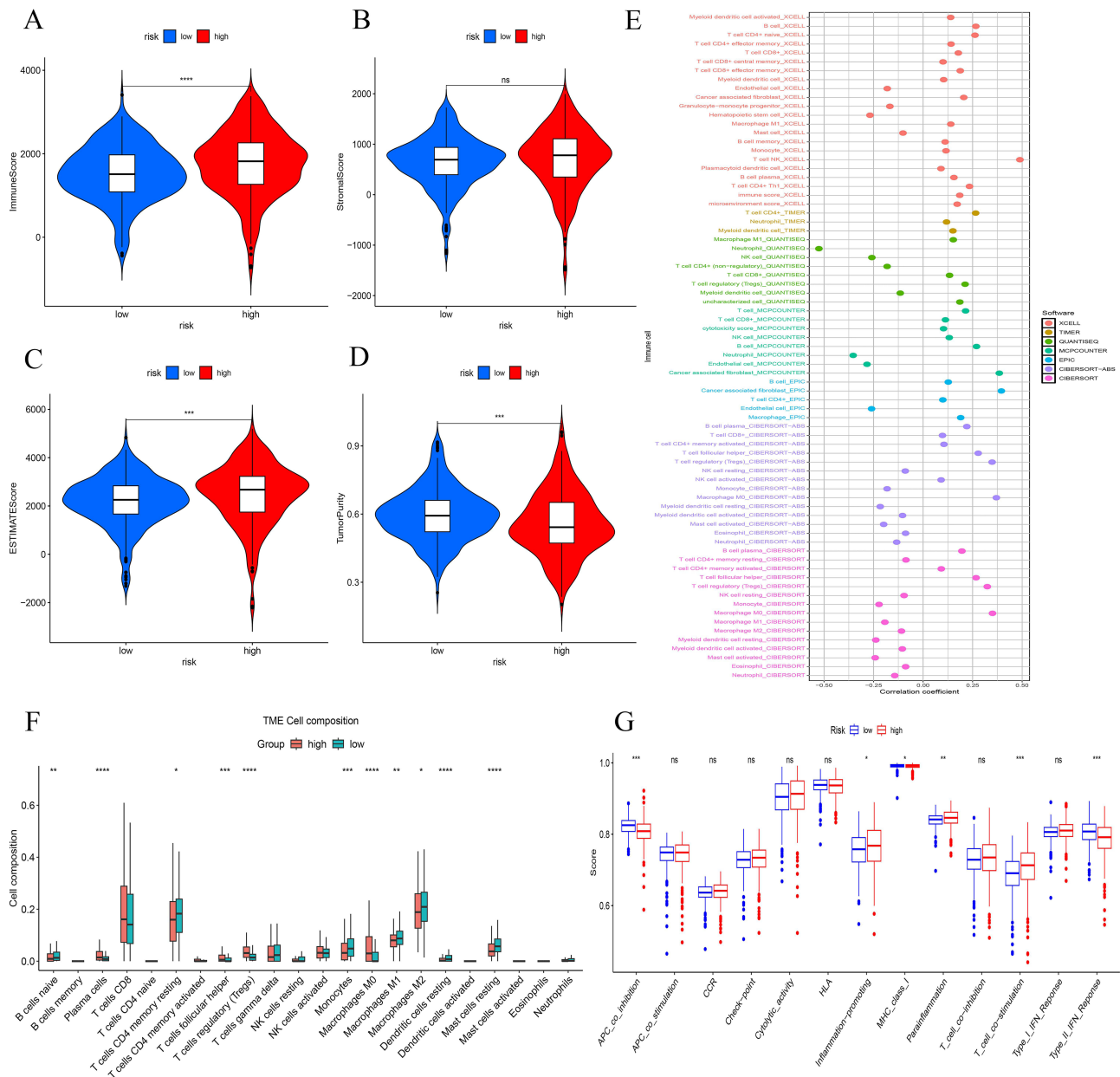


Figure 8 Analysis of tumor immune microenvironment. Violin plots of differences in immune scores (A), stromal scores (B), ESTIMATE scores (C), and tumor purity (D) for different risk subgroups; Bubble plots of correlations between immune cells and risk scores under six algorithms (E); Proportions of 22 immune cells in two subgroups under the CIBERSORT algorithm (F); single sample gene set EA (G). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: not significant.

RT-qPCR and HPA

We obtained protein expression images of LRP-related genes in KIRC cells and normal cells from the HPA database (Figure 9A). RT-qPCR outcome displayed that only TMEM74B expression was insignificant. The IYD and QRFPR were highly expressed in normal tissues, while the remaining 3 mRNAs (CEL, C1QTNF6, IL20RB) were highly expressed in tumor tissues (Figure 9B).

External Cohort Validation of LRPMRs

To further verify the accuracy of this model, we employed E-Mc as the independent validation cohort. We calculated the risk scores for patients in this cohort with same method applied to the TCGA cohort. In the E-Mc, H-R patients showed poorer survival outcomes compared to those in the L-R group (Figure 10A). We further validated the prognostic

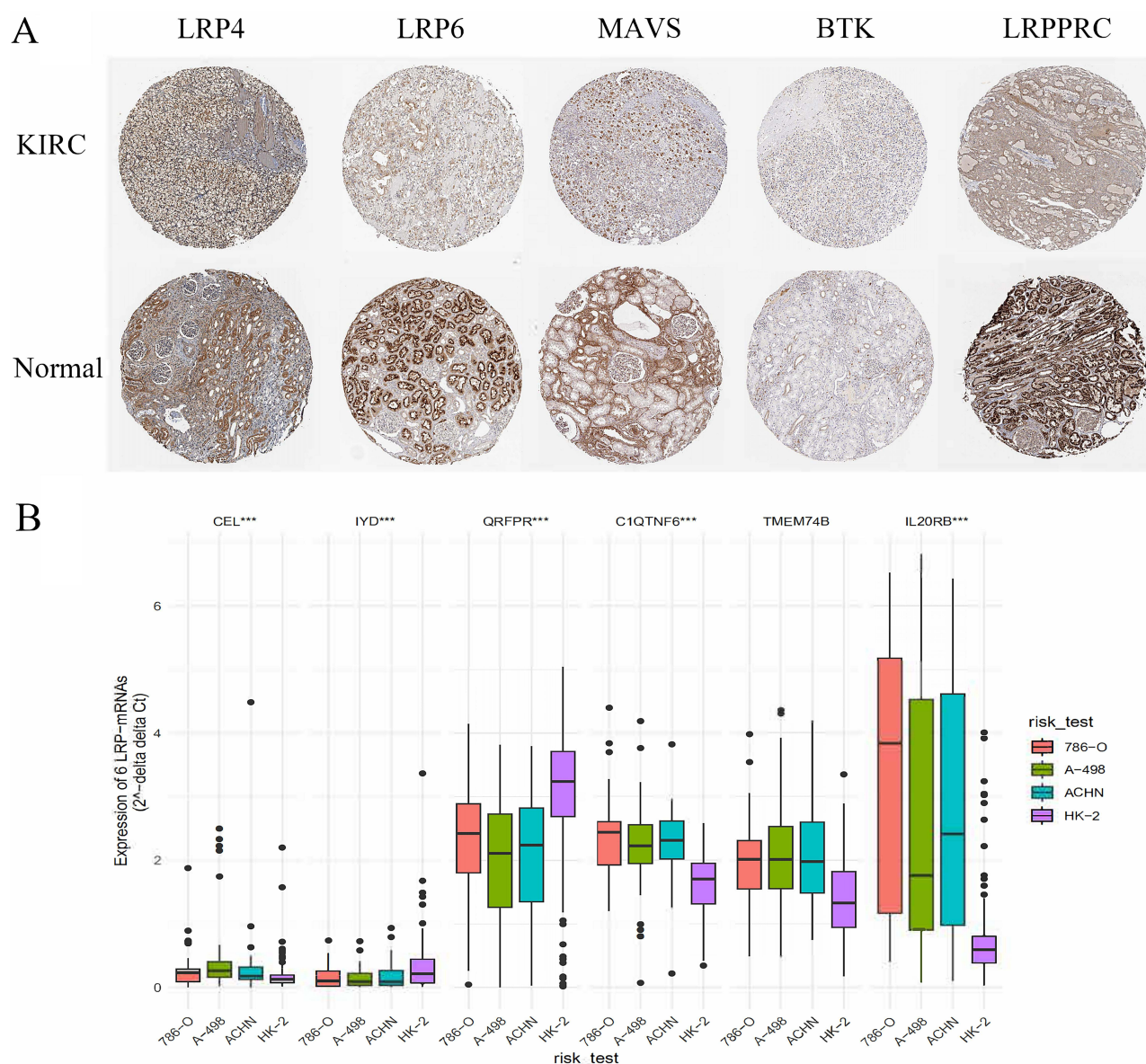


Figure 9 In vitro experimental validation of the risk model. Immunohistochemical staining images of partial LRP-associated gene proteins in KIRC tissue and normal tissue (**A**); Relative expression of 6 LRPMRs in different risk subgroups (**B**). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

significance of LRPMRs through both uCoxRA and mCoxRA (Figure 10B and E). The correlation between risk scores and clinical factors (age, sex, tumor grade, and stage) was evaluated using chi-square tests (Figure 10C). ROC analysis revealed AUC values for LRPMRs of 0.811 for one-, 0.789 for three-, and 0.797 for five-year (Figure 10D). Additionally, we observed 6 LRPMRs differential expression levels between the H-R and L-R groups (Figure 10F).

Discussion

In recent years, there has been some progress in the diagnostic imaging and clinical treatment of KIRC, but the prognosis of patients is still poor, especially in the advanced or metastatic stage, and the 5-year survival rate is still low.²⁴ Additionally, the complex molecular mechanisms and TME underlying KIRC progression, treatment resistance, and prognosis are not yet fully understood, posing significant challenges to clinical management.²⁵ To address these gaps, we developed a prognostic model based on LRPMRs. The model demonstrated a significant reduction in OS for H-R patients compared to L-R patients. Integrating risk model and clinical factors, including age, gender, and stage, into the

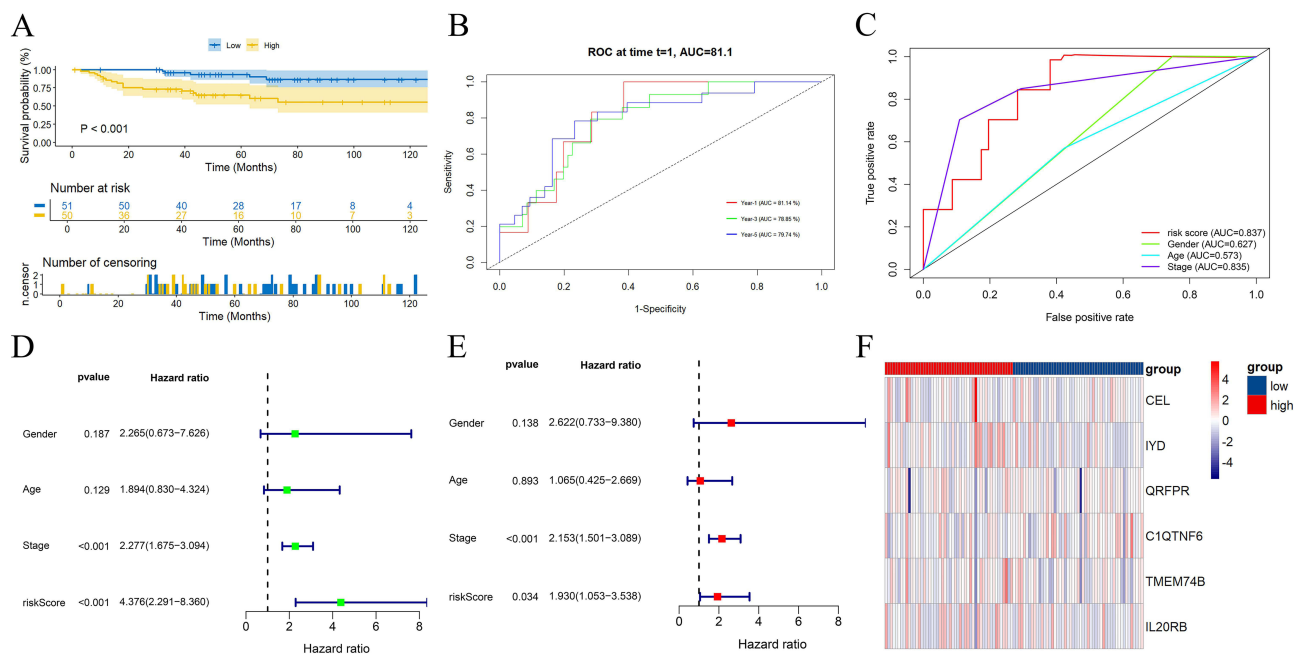


Figure 10 Validation of LRPMRs in Independent External Cohorts. Survival curves of patients (A). TdROC curves (B). ROC curves containing different clinical information (C). Univariate and multivariate independent prognostic analysis (D and E). Heat map of 6 LRPMRs expressions (F).

nomogram enhances its predictive performance. Mechanistic analysis of TMB and immune microenvironment characterization provides insights into the biological basis of risk model. Furthermore, drug susceptibility analysis identified customized treatment regimens for H-R and L-R populations, highlighting the clinical relevance and applicability of this model. Finally, RT-qPCR analysis was conducted to validate the difference in the expression of the mRNAs in normal cell and tumor cell samples.

In our research, we developed a LRPMRs-based predictive framework and a corresponding nomogram, which demonstrated strong predictive performance validated through multiple assessment tools, including the ROC curve, C-index, and calibration curve. The LRPMRs risk model we constructed exhibited higher AUC values, outperforming the KIRC model studied by Xu et al.²⁶ Previous studies have indicated that among the 6 genes screened out finally, IYD is downregulated in triple-negative breast cancer, correlating with worse survival outcomes in affected individuals.²⁷ C1QTNF6 was upregulated in tumor tissues, and its knockdown led to cell division arrest at the G2/M checkpoint in CaL27 and SCC-9 cell lines, while also promoting apoptosis, thereby contributing significantly to tumor development.²⁸ IL20RB expression is significantly upregulated in cancer tissues and is linked to poor prognosis.²⁹ These findings may enhance the predictive value of risk scores. In conclusion, our LRPMR-based prognostic model exhibits superior predictive accuracy in KIRC patients, surpassing traditional evaluation approaches.

We further investigated the underlying mechanisms of action and related functions. GSEA highlighted several pathways related to proximal tubule transport that were predominantly enriched in the L-R group. The proximal tubule transport plays an important role in maintaining kidney function and systemic metabolic balance.^{30,31} Dysfunction causes the transcription factor HIF-1 α to be activated under hypoxic conditions, promoting the adaptation of tumor cells to the harsh environment.³² In the H-R subgroup, BCR signaling is altered by co-receptors like CD22, contributing to the development of B cell malignancies.^{33,34} Immune function analysis revealed differences in functions such as APC co-suppression, type II IFN responses and T cell co-stimulation between two risk groups. Previous research has indicated that IFN- γ serves as a reliable predictor of response to cancer immunotherapy and may play a role in limiting tumor progression.³⁵ A higher TMB is often linked to a poorer prognosis in KIRC patients.³⁶ Inactivation of tumor suppressor genes, such as VHL, has been implicated in cancer progression. Notably, loss of VHL function has been shown to promote histone lactylation, which activates the transcription of platelet-derived growth factor receptor β (PDGFR β),

thereby driving KIRC development.³⁷ These findings provide a valuable foundation for further in-depth research on the molecular mechanisms and potential therapeutic targets of KIRC progression in the future.

The clinical utility of this prognostic model in guiding treatment selection was further assessed in our study. In immune cell infiltration assays, high levels of T follicular helper (Tfh) cell infiltration were associated with better prognosis, as Tfh cells enhance anti-tumor immunity by supporting B cells in producing tumor-targeting antibodies.^{38,39} These antibodies can either attack tumor cells directly or facilitate their recognition by the immune system.⁴⁰ Similarly, M1 macrophages promote anti-tumor responses, and resting mast cells correlate with improved survival in KIRC patients.⁴¹ The L-R subgroup's enrichment in these immune cells may contribute to their prolonged survival. Conversely, regulatory T cells (Tregs) in H-R patients suppress immune responses by inhibiting co-stimulatory signals (eg, CD80/CD86 via CTLA4) and secreting inhibitory cytokines, creating an immunosuppressive environment that worsens prognosis.⁴² Our findings are consistent with recent studies demonstrating the role of immune-related molecular signatures in KIRC prognosis.⁴³ Notably, differences in Tregs, M1 macrophages, and T follicular helper cells between high- and low-risk groups further support the relevance of immune microenvironment heterogeneity to clinical outcomes and responsiveness to immune checkpoint therapies.⁴⁴ These immune cells offer a direction for immunotherapy. In the drug susceptibility analysis, the H-R group showed increased responsiveness to therapeutic agents that inhibit the PI3K/mTOR signaling pathway, including dactolisib. Dysregulation of PI3K/AKT/mTOR and RTK signaling pathways are commonly observed in KIRC and drives tumor progression.⁴⁵ Tyrosine kinase inhibitors (TKIs) like sunitinib and sorafenib remain first-line therapies, but their effectiveness is often hindered by acquired resistance and metastasis.^{46,47} Resistance mechanisms, such as increased GLUT1 and MDR1 expression, pose significant challenges. Staurosporine (STS), by inhibiting MDR1 and GLUT1, may overcome TKI resistance and enhance the efficacy of RTK-targeted therapies.^{48,49} This suggests a synergistic role for STS in managing drug-resistant or metastatic KIRC. Overall, this model offers valuable insights into the immune landscape and drug responsiveness of KIRC, providing a foundation for more tailored and effective therapeutic strategies.

Our study offers several notable advantages. To evaluate the applicability of our model, we compared it to previous studies. For example, although Yin et al's model based on autophagy-related genes demonstrated its predictive value in acting on KIRC we broadened its application by exploring its role in selecting antitumor drugs.⁵⁰ Furthermore, our model demonstrated superior performance in forecasting the 5-year patient survival probability (AUC = 0.82) relative to Wu et al's model (AUC = 0.74).⁵¹ Furthermore, we further validated the accuracy of our model using external datasets, the step not included in Cai et al's study.⁵² We also performed more detailed antitumor susceptibility analysis, which provided valuable insights into KIRC treatment. However, we acknowledge that our studies were flawed due to limitations in data sources and the lack of clinical trials for drug sensitivity analysis.

Conclusion

Our risk model and nomogram based on six LRPMRs showed strong predictive performance for patient outcomes. Higher TMB and CD22-mediated BCR regulation and FCGR pathway activation in the H-R group may predict a worse prognosis. Furthermore, H-R group may exhibit increased sensitivity to drugs targeting the RTK and PI3K/MTOR pathways. However, further basic research is needed to fully explore these underlying mechanisms. In conclusion, the LRPMRs signature represents a promising prognostic biomarker for KIRC, with implications for individualized treatment. Future prospective clinical trials are warranted to validate this model and accelerate its translation into clinical practice.

Abbreviations

AUC, Area Under the Curve; AJCC, American Joint Committee on Cancer; APC, Antigen-presenting Cell; C-index, Concordance Index; DCA, Decision Curve Analysis; EA, Enrichment Analysis; E-Mc, E-MTAB-1980 Cohort; GSEA, Gene Set Enrichment Analysis; HPA, Human Protein Atlas; HR, Hazard Ratios; High-risk, H-R; IC50, Half Maximal Inhibitory Concentration; KEGG, Kyoto Encyclopedia of Genes and Genomes; KIRC, Kidney Renal Clear Cell Carcinoma; K-M, Kaplan-Meier; LASSO, Least Absolute Shrinkage and Selection Operator; Low-risk, L-R; LRP, Low-Density Lipoprotein Receptor-Related Protein; LRPMRs, LRP-related mRNAs; mCoxRA, Multivariate Cox Regression Analysis; mRNA, Messenger RNA; Nomogram, Predictive Statistical Model; OS, Overall Survival; PCA, Principal Component Analysis; PPI, Protein-protein

Interaction; ROC, Receiver Operating Characteristic; STS, Staurosporine; ssGSEA, Single-sample Gene Set Enrichment Analysis; TAM, Tumor-associated Macrophage; TCGA, The Cancer Genome Atlas; TdROC, Time-dependent ROC Curve; TIMER, Tumor Immune Estimation Resource; TME, Tumor Microenvironment; TMB, Tumor Mutation Burden; TNM, Tumor-node-metastasis; TIDE, Tumor Immune Dysfunction and Exclusion; uCoxRA, Univariate Cox Regression Analysis.

Data Sharing Statement

Data is provided within the manuscript or supplementary information files.

Ethics Approval and Consent

This article does not contain any studies with human participants or animals performed by any of the authors. This study utilized publicly available data from The Cancer Genome Atlas (TCGA) database, which has been ethically approved by the respective institutions. As the data is anonymized and does not involve direct human intervention, the study was exempt from requiring institutional review board (IRB) approval based on national legislation. According to Article 32 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects (February 18, 2023, China), studies involving publicly available and de-identified data do not require IRB approval.

Informed Consent

For this type of study formal consent is not required.

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Author Contributions

Zhihong Liu had full access to all the data in the manuscript and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

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Disclosure

The authors certify that there is no conflict of interest regarding this work.

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