



LAC-TME classifier: machine learning-driven model predicts survival and prioritizes targeted therapy in clear cell renal cell carcinoma

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Abstract

Background Clear cell renal cell carcinoma (ccRCC) is a major type of kidney cancer, making up about 80% of cases, with advanced stages showing low survival rates. Current treatments face challenges like toxicity and drug resistance. Studies indicate lactate, through the Warburg effect, promotes an immune-suppressive tumor microenvironment (TME), prompting the development of the LAC-TME classifier using machine learning to predict outcomes and personalize treatment.

Methods The study used data from TCGA-KIRC set and E-MTAB-1980 set, analyzing gene expression, mutations, and clinical data. It employed differential expression analysis, immune infiltration assessment, and 101 machine learning algorithms to build the classifier, integrating lactate-related genes and TME features, with predictive capability verified.

Results The LAC-TME classifier, constructed by integrating 9 lactate-related differentially expressed genes and TME cells, demonstrated high predictive accuracy (C-index of 0.92 in the training set and 0.73 in the validation set). Patients were categorized into three groups: Lactate^{low} + TME^{low} (best prognosis), Lactate^{high} + TME^{high} (poorest prognosis), and a mixed group. This classifier can predict 1- to 5-year survival rates, with an AUC of 0.88–0.92. Notably, the Lactate^{high} + TME^{high} subgroup was associated with immunosuppression and poor response to immunotherapy. As the core lactate-related gene of the LAC-TME classifier, the knockdown of LGALS1 significantly inhibits the proliferation and migration of ccRCC cells, verifying the biological rationality of the classifier.

Conclusion The LAC-TME classifier, integrating metabolic and immune data, offers a new tool for ccRCC prognosis and treatment guidance. Further validation is needed to confirm its clinical potential, reflecting the ongoing need for robust medical research.

Keywords Clear cell renal cell carcinoma · Lactate · Tumor microenvironment · Machine learning · LGALS1

Introduction

As a prevalent urological malignancy, renal cell carcinoma (RCC) was associated with more than 431,000 incident cases and 179,000 mortality events worldwide in 2020 (Sung et al. 2021). Clear cell renal cell carcinoma

(ccRCC) represents the most prevalent histological subtype of RCC, constituting approximately 80% of all RCC cases (Hsieh et al. 2017). Even when detected early, metastatic disease is observed in nearly 21% of patients during primary diagnosis (Dabestani et al. 2016). Furthermore, 25–40% of patients with localized ccRCC experience recurrence and metastasis following standard treatment with radical or partial nephrectomy (Young et al. 2024). Consequently, the 5-year survival rate for advanced ccRCC remains critically low at 11.7% (Siegel et al. 2017). The current first-line protocols utilizing programmed death-1 (PD-1) inhibitors combined with Vascular Endothelial Growth Factor Receptor (VEGFR)-targeted agents or Cytotoxic T-Lymphocyte-Associated Protein 4 (CTLA-4) inhibitors demonstrate substantial survival benefits in advanced ccRCC (Young et al. 2024). However, significant controversies persist

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in first-line treatment selection, especially when managing patients with severe toxicities or primary/secondary resistance (Makhov et al. 2018). Therefore, early and accurate patient stratification, prediction of treatment responses, and identification of new molecular markers can enhance patient prognosis and enable effective individualized disease management. Metabolic dysregulation is considered a potential etiological factor in tumorigenesis. The hallmark Warburg effect in tumors is characterized by excessive glucose uptake and substantial lactate production (Courtney et al. 2018; Liao et al. 2024). Historically dismissed as tumor waste, Warburg effect-derived lactate is now understood to act as a bioenergetic signaling molecule that traverses cellular boundaries, emerging as a critical communicator between neoplastic and stromal cells within the tumor microenvironment (TME) (Wang et al. 2020). Lactate overload from malignancies induces TME acidosis, facilitating Myeloid-derived suppressor cells (MDSCs) expansion and functional suppression of cytotoxic lymphocytes, thereby establishing an immunosuppressive niche conducive to tumor survival (Li et al. 2022). The TME harbors diverse innate immune populations that critically regulate cancer pathogenesis (Prenen and Mazzone 2019; Frauwirth et al. 2002) (Husain et al. 2013). High lactate levels inhibit lactate extrusion in T lymphocytes, compromising cytotoxic T cells (CTLs) expansion through metabolic interference (Zhang et al. 2025a, 2025b; Ippolito et al. 2019; Fischer et al. 2007). Acidic TME conditions and lactate accumulation induce M2 macrophage polarization, triggering immunosuppressive signaling that enhances tumor invasion and neovascularization (Ippolito et al. 2019) (Hughes et al. 2015). Lactate-mediated TME acidification exerts dual inhibitory effects on NK cells – direct functional impairment and indirect suppression via myeloid network disruption (Brand et al. 2016). Collectively, lactate modulates TME immune responses through both proton-dependent and lactate-specific signaling axes. Deciphering the coordinated interaction mode between lactate metabolism and the TME in ccRCC could provide mechanistic insights into clinical response variability and identify actionable therapeutic vulnerabilities.

Leveraging multi-omics clinical cohorts (TCGA-KIRC and E-MTAB-1980), we systematically mapped lactate-driven TME dynamics in ccRCC using a computational framework of 101 machine learning algorithms. The resultant LAC-TME Classifier emerged as a robust prognostic tool for clinical outcome prediction, demonstrating utility in guiding therapy selection for immunotherapy-sensitive subgroups. Mechanistically, *LGALS1* was prioritized as the principal lactate-related determinant, with functional validation revealing its central role in ccRCC pathogenesis.

Methods

Acquisition of datasets and gene sets

The TCGA-KIRC cohort containing FPKM values, mutation profiles, and clinical metadata was retrieved from UCSC Xena Browser and cBioPortal database. The independent validation cohort E-MTAB-1980 with gene expression matrices and survival records was acquired from ArrayExpress repository. The lactate-related gene set was systematically curated through manual screening of published literature (Supplementary Table 1). All FPKM values underwent standardized conversion to transcripts per million (TPM) followed by $\log_2$ -transformed normalization.

Gene expression differential analysis

The differential expression analysis workflow was implemented with limma package (version 3.60.6) incorporating RNA-seq specific adaptations. During preprocessing, low-expression genes were filtered from TPM-normalized data using a threshold of $\text{TPM} \geq 0$ in $\geq 50\%$ samples. Sequencing depth and heteroscedasticity were corrected using voom function. Linear model fitting via lmFit was performed prior to empirical Bayes moderation using the eBayes method for estimating significance. Significant differentially expressed genes were identified using thresholds of $|\log_2 \text{ fold change}| \geq 1.5$ and FDR-adjusted $p\text{-value} < 0.05$. The complete analytical pipeline was executed in R statistical computing environment (version 4.1.2) with Bioconductor dependencies.

Immune infiltration analysis

The xCell algorithm was applied to both training and validation cohorts using xCell (v1.1.0) to calculate enrichment scores for 64 immune and stromal cell types. Input data underwent automatic validation with removal of extremely low-expressed genes, ultimately producing Min–Max normalized cellular infiltration scores (0–1) that were further filtered by univariate Cox analysis. CIBERSORT deconvolution: Immune cell composition was analyzed using the CIBERSORTx web portal with the predefined LM22 immune cell signature matrix. Parameter settings included 1000 permutations to output immune cell subset proportions (sum = 100%).

LAC-TME classifier construction

The Mime1 package (version 0.0.0.9000) was employed to systematically assess 101 machine learning algorithm

combinations through comprehensive evaluation of differentially expressed genes and xCell-quantified cell populations demonstrating univariate prognostic significance. Model selection was performed by computing the mean Harrell's concordance index (C-index) across all algorithm combinations, with optimal model ensemble identified through maximal C-index criteria. The randomForestSRC package's `rfsrc` function was implemented for risk score modeling with parameters set as follows: `ntree=1000`, `nodesize=5`, `mtry=3`, `nodedepth=-10`, and `nsplit=10`. Model predictions were generated via `predict(fit, newdata) $ predicted`, producing continuous risk scores that quantify individual patient prognosis. Patients were stratified into high- and low-risk groups using the mean RS value as the cutoff.

mFuzz

Using the mFuzz package (v2.64.0) to analyze gene expression dynamics in three prognostic subgroups (optimal/intermediate/poor) defined by the LAC-TME classifier. Subgroup-specific expression matrices were constructed by calculating mean expression values for each gene across defined prognostic strata (Lactate^{low}+TME^{low}, Mixed, Lactate^{high}+TME^{high}). Genes exhibiting zero standard deviation across subgroups were filtered using `filter.std` function. Z-score normalized genes were subjected to Fuzzy C-Means clustering (cluster number $c=6$, fuzzifier parameter $m=5.3$) to determine membership coefficients, where genes with membership ≥ 0.5 were designated as core cluster constituents. Cluster patterns were visualized through composite plots containing smoothed trend lines (`ggplot2`) and expression gradient heatmaps (`RColorBrewer`), implementing perceptually uniform color scales for biological interpretation.

Enrichment analyses

The `c2.cp.kegg.v7.4.symbols.gmt` and `c2.cp.reactome.v7.4.symbols.gmt` gene set collections were retrieved from the Molecular Signatures Database (MSigDB) to establish pathway reference backgrounds. Gene clusters were functionally annotated through enrichment analysis using `clusterProfiler` package (v3.14.3), which aligns cluster-specific genes with predefined pathway databases. Analytical parameters were configured with gene set size constraints ($5 \leq \text{genes} \leq 5000$) to ensure biological relevance. Pathway enrichment significance was determined using dual thresholds (nominal $p < 0.05$; false discovery rate < 0.1) for rigorous multiple testing correction. Cluster-specific core genes (top 50 membership-weighted genes) underwent single-sample gene set enrichment analysis (ssGSEA) through BEST algorithm (Liu et al. 2023) to quantify pathway

activity scores. Patients were stratified into subgroups using ssGSEA activity scores for Kaplan–Meier survival curve generation and log-rank testing. Pre-ranked gene set enrichment analysis (GSEA) was conducted using the `fgsea` package (v1.28.0) on HALLMARK gene sets, leveraging differentially expressed genes identified between the Lactate^{low}+TME^{low} and Lactate^{high}+TME^{high} subgroups within the LAC-TME classifier training cohort. Genes were pre-sorted by log₂ fold change values in descending order, with parameter settings of minimum gene set size (`minSize=5`) and statistical significance threshold ($\text{FDR} < 0.05$). Gene Ontology Biological Process (GO-BP) enrichment analysis was separately conducted for up- and down-regulated differentially expressed genes using the `enrichGO` function, applying identical significance thresholds ($\text{FDR} < 0.05$). The regulatory direction of signaling pathway activity was assessed using the `run_mlm` function in `decoupleR` package (v3.6.0), incorporating log₂ fold change values from differentially expressed genes. The input matrix containing log₂ fold change values from differential expression analysis (including up/down-regulated gene sets after filtering low-expression genes) was integrated with the Progeny pre-compiled pathway network (human model, top 500 genes per pathway) to construct a gene-pathway weight matrix. Pathway interactions were filtered to retain those containing a minimum of 5 genes (`minSize=5`). Composite activity scores were computed for each pathway followed by FDR correction using a threshold of < 0.1 .

TIDE tumor immune dysfunction and exclusion analysis

Tumor Immune Dysfunction and Exclusion (TIDE) algorithm was implemented to assess immunotherapy response potential in TCGA-KIRC cohort. The log₂(TPM+1)-transformed normalized expression matrix was subjected to low-expression filtering (genes with $\text{TPM} \geq 1$ across all samples retained) and subsequently processed through the TIDE computational framework (<http://tide.dfci.harvard.edu/>). This generated three key metrics per sample: 1) Immune Dysfunction score quantifying T cell exhaustion level; 2) Immune Exclusion score measuring T cell infiltration barriers; and 3) Composite TIDE score with predefined clinical threshold of 1 (TIDE < 1 classified as immunotherapy-sensitive subgroup).

Drug sensitivity prediction

Based on the GDSC drug database of pRRophetic package (v1.4.0), the IC₅₀ values of drugs were predicted from the normalized gene expression matrix of tumor samples. Ridge regression algorithm (lambda auto-optimization)

was used to predict the IC₅₀ values of Sunitinib, Temsirolimus, Rapamycin and Rapamycin by fixing the random seed (12,345) with the “allSolidTumors” organizational model. Rapamycin and Sorafenib using a ridge regression algorithm (lambda auto-optimization).

Cell culture and cell transfection

ccRCC cell lines 786-O and 769-P were selected for experimentation. The cultivation of both cell lines was undertaken in RPMI-1640 medium, which was enriched with 10% foetal bovine serum and 1% penicillin–streptomycin antibiotic solution. The cells were then placed within an incubator set at 37 °C, with the presence of 5% CO₂. Twenty-four hours prior to the transfection process, a total of 1.5×10^5 cells were distributed evenly across a total of six 6-well plates. The first stage of the process is to configure the siRNA master mix at a concentration of 20 µM. Next, an appropriate amount of the master mix is added to 200 µL of jetPRIME® buffer to achieve a final concentration of 45 µM of siRNA per well. Following this, 4 µL of jetPRIME® reagent is added. The mixture is then incubated for 15 min at room temperature, after which it is added to the 6-well plate with pre-layered cells. Cellular RNA was extracted 24 h after transfection, and cellular proteins were extracted 48 h later for subsequent experimentation. siRNA was provided by Tsingke Biotechnology (TSGXS101Homo); sequences are detailed in Supplementary Table 2. siNC is nonsense sequence small interfering RNA.

Quantitative real-time PCR (qPCR) and western blot

Cellular RNA was extracted using the Novozymes FastPure Complex Tissue/Cell Total RNA Isolation Kit (RC113–01), and the procedure was strictly followed according to the kit instructions. The extracted RNA was converted to cDNA by reverse transcription reaction using Takara PrimeScript™ RT reagent Kit (RR037Q). qPCR reaction solution was configured using Takara TB Green® Premix Ex Taq™ II and detected by LightCycler480 instrument. ACTIN was used as the reference gene, with relative expression calculated via the $\Delta\Delta C_t$ method. The primer sequences are detailed in Supplementary Table 2. Cells were lysed using RIPA lysate (P0013B) and the protease inhibitors cocktail (HY-K0010) and PSMF (ST507) were added to prevent protein degradation. The extracted protein samples were separated by SDS-PAGE gel electrophoresis and transferred to PVDF membrane. Selleck (F1039) was used for LGALS1 antibody at a dilution of 1:1000, and proteintech (11,224–1-AP) was used for α -TUBULIN antibody at a dilution of 1:5000. After overnight incubation at 4 °C, washing and

color development, the expression levels of the target proteins were detected by chemiluminescence imaging system.

Cell function assay

Cell proliferation ability was detected by CCK8 assay (C0037), 2×10^3 cells were inoculated in each well, and the first absorbance value was measured after 6 h of incubation with CCK8 reagent for 4 consecutive measurements. Cell migration and invasion were detected by Transwell assay and scratch assay, respectively. In the Transwell assay, 3×10^5 cells were suspended in serum-free medium and added into the upper chamber of Transwell, and 15% fetal bovine serum was added into the lower chamber, and the migrated cells were counted after 24 h of incubation; in the invasion assay, the cells were counted in the upper chamber of Transwell, and the migrated cells were counted after 24 h of incubation. For the invasion experiment, the upper chamber of the Transwell was pre-coated with Matrigel (354,277), and the same number of cells was added and incubated for 24 h, then the invading cells were counted. In the scratch assay, when the cells grew to 90% confluence, a 200 µl sterile tip was used to make a scratch, which was observed and photographed at 0 and 12 h, respectively, and the width of the scratch was measured to evaluate the migration ability. The cell cycle assay was performed using a cell cycle kit (70-CCS012), and the collected cells were stained and analyzed by flow cytometry according to the instructions to obtain the proportion of cells in each cell cycle stage.

Statistical analysis

For categorical variables, chi-square tests or Fisher's exact tests were used. For continuous variables, the Shapiro–Wilk test was conducted to assess normality. Then, based on the results, t-tests (parametric) or Wilcoxon/Kruskal–Wallis tests (non-parametric) were applied. For multiple group comparisons, Dunn's post-hoc test was carried out. Survival analysis was performed via Kaplan–Meier curves (using the log-rank test) and Cox regression (both univariate and multivariate). A significance level of $P < 0.05$ was applied throughout.

Results

Construction of a LAC-TME prognostic classifier via machine learning

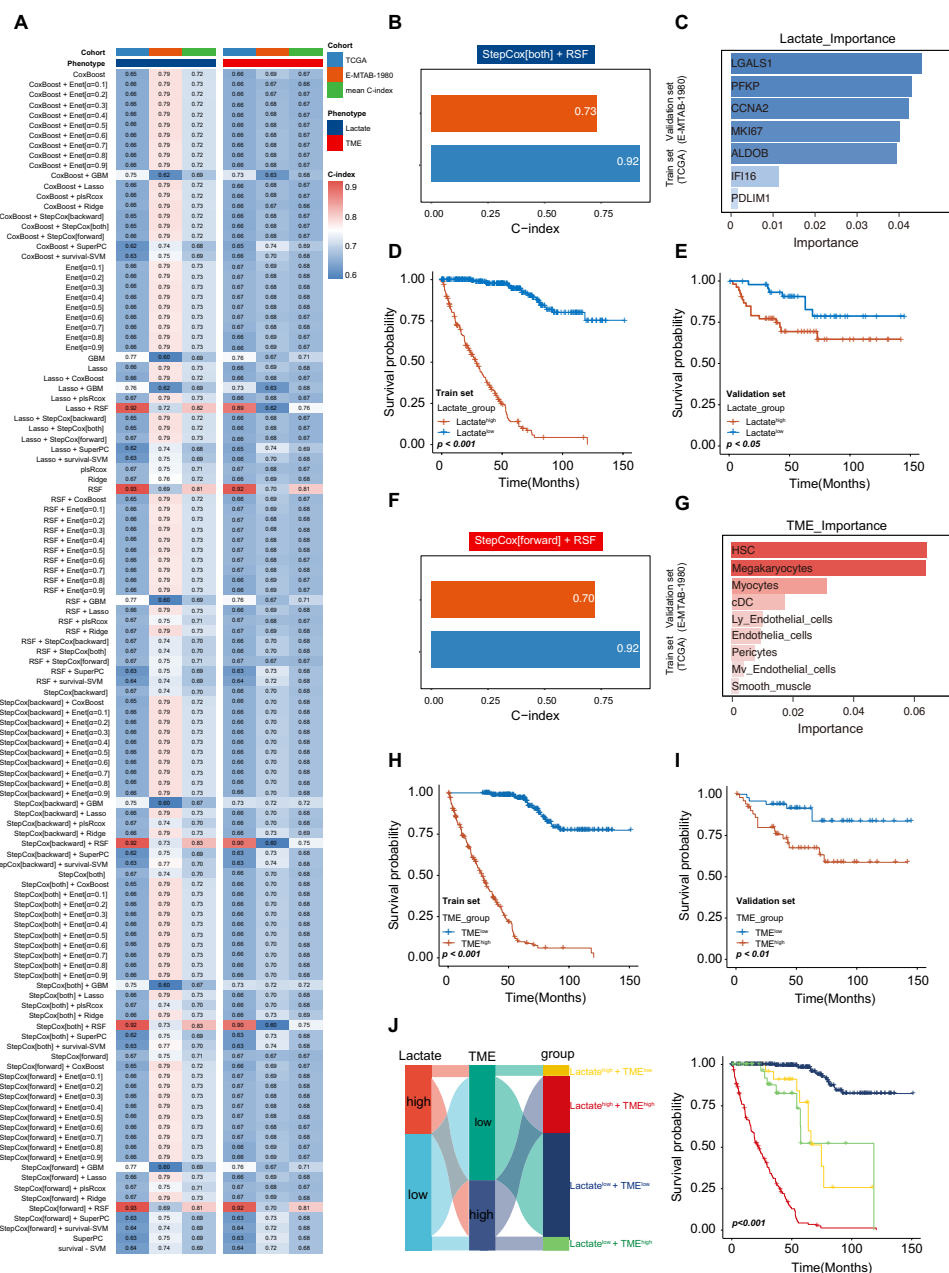
Differential expression analysis was performed in TCGA-KIRC to compare RCC with normal tissue, using thresholds

of $|\log_2FC| > 1.5$ and adjusted $p < 0.05$. This identified 2,714 differentially expressed genes (DEGs), with 2,000 upregulated and 714 downregulated. Intersection with a lactate-related gene set yielded 16 DEGs, including 9 upregulated and 7 downregulated genes. These 16 genes were subjected to 101 machine learning algorithms using the Mimel package (Fig. 1A), with TCGA-KIRC as the training cohort and E-MTAB-1980 as the validation cohort. Based on the concordance index (c-index) values and their means in the validation cohort, the StepCox[both]+RSF model was selected, achieving c-index values of 0.92 and 0.73 in the training and validation cohorts, respectively (Fig. 1B). The model incorporated seven genes: *LGALS1*, *PFKP*, *CCNA2*,

MKI67, *ALDOB*, *IFI16*, and *PDLIM1* (Fig. 1C). Patients were stratified into Lactate^{high} and Lactate^{low} groups based on risk scores above or below the mean. Kaplan–Meier (KM) survival analysis revealed significantly worse overall survival (OS) in the Lactate^{high} group compared to the Lactate^{low} group in both cohorts (Fig. 1D, E).

Next, immune and stromal cell infiltration levels were assessed in the training and validation cohorts using xCells. The Mimel package was employed to analyze these cell components with 101 machine learning algorithms (Fig. 1A). The StepCox[forward]+RSF model was selected, with c-index values of 0.92 and 0.70 in the training and validation cohorts, respectively (Fig. 1F). This model

Fig. 1 Construction of a LAC-TME Prognostic Classifier via Machine Learning: **A** C-index heatmap of machine-learning algorithms via the Mimel package. **B** Histogram showing C-index of optimal prognostic model for lactate-related gene. **C** Histogram showing the importance of the optimal prognostic characterization of lactate-related genes. **D** Kaplan–Meier overall survival plots for training (n=522) and **E** validation (n=101) sets stratified by the training set's mean lactate risk score. **F** Histogram showing C-index of optimal prognostic model for TME. **G** Histogram showing the significance of the optimal prognostic characterization of TME. **H** Kaplan–Meier overall survival plots for training (n=522) and **I** validation (n=101) sets stratified by the training set's mean TME risk score. **J** Sankey diagram (left) illustrating the relationship between lactate-related gene expression, TME features, and LAC-TME classifier subgroups in the TCGA-KIRC cohort (n=522). The left axis represents lactate risk grouping, the middle axis represents TME risk grouping, and the right axis represents LAC-TME classification results. Kaplan–Meier overall survival curves of training cohorts stratified into four subgroups by the LAC-TME classifier (right)



included nine cell components, including hematopoietic stem cells (HSCs) (Fig. 1G). Patients were classified as TME^{high} or TME^{low} based on risk scores above or below the mean, consistent with prior findings linking higher immune scores to poorer prognosis in ccRCC. KM survival analysis confirmed significantly worse OS in the TME^{high} group compared to the TME^{low} group in both cohorts (Fig. 1H, I).

Finally, we integrated lactate and TME groupings to develop the LAC-TME classifier (Fig. 1J). The Lactate^{high}+TME^{high} subgroup exhibited the poorest prognosis, while the Lactate^{low}+TME^{low} subgroup showed the best prognosis (Fig. 1K).

Validation and molecular characterization of the LAC-TME classifier

To further evaluate the LAC-TME classifier, we combined the Lactate^{high}+TME^{low} and Lactate^{low}+TME^{high} subgroups into a single "Mixed" category, forming a tripartite LAC-TME classifier. KM survival analysis demonstrated robust prognostic value in both the training and validation cohorts (Fig. 2A, B). The classifier achieved area under the curve (AUC) values for predicting 1-, 3-, and 5-year survival rates of 0.88 (95% CI: 86.34–90.33), 0.90 (95% CI: 86.45–92.80), and 0.92 (95% CI: 89.14–95.67), respectively (Fig. 2C). Notably, the classifier maintained strong predictive performance across subgroups stratified by age, sex, tumor grade, and tumor stage (Fig. 2D).

We next examined tumor mutational burden (TMB) across the three groups. The Lactate^{high}+TME^{high} group, with the poorest prognosis, exhibited the highest TMB, while the Lactate^{low}+TME^{low} group had the lowest (Fig. 2E). Additionally, genes associated with cell cycle and proliferation, including *CDKN2A*, *CDKN2B*, *CDKN2B-AS1*, *CDKN2A-AS1*, *NEGR1*, *PTPRD*, and *PTEN*, showed significantly higher deletion frequencies in the Lactate^{high}+TME^{high} group compared to the other groups (Fig. 2F). Waterfall plots illustrated the top 10 mutated genes in the Lactate^{high}+TME^{high} and Lactate^{low}+TME^{low} groups (Fig. 2G, H). Notably, *BAP1* mutation frequency was significantly elevated in the Lactate^{high}+TME^{high} group compared to the Lactate^{low}+TME^{low} group (Fig. 2I). KM survival analysis further revealed that *BAP1* mutations were associated with worse OS (Fig. 2J), consistent with prior studies linking *BAP1* mutations to aggressive ccRCC phenotypes and metabolic dysregulation (Langbein et al. 2022).

Functional and pathway analysis of the LAC-TME classifier

Using the Mfuzz package, genes in the LAC-TME classifier were clustered into 6 distinct clusters. Clusters 3 and 5

Fig. 2 Validation and Molecular Characterization of the LAC-TME Classifier: **A** Kaplan–Meier overall survival curves of the train (n=522) set and **B** independent validation (n=101) set stratified into three different subgroups based upon the LAC-TME classifier. **C** ROC curves for 1-, 3-, and 5-year survival prediction of the train set based on the LAC-TME classifier. **D** Prognostic Kaplan–Meier curves of the LAC-TME classifier stratified by age, sex, tumor grade, and tumor stage. **E** Violin plot of TMB differences among three subgroups classified by the LAC-TME classifier (n=319). **F** Bar plot of gene deletion frequency differences among three subgroups classified by the LAC-TME classifier. **G** The OncoPrint was constructed by the top 10 mutation genes between Lactate^{high}+TME^{high} and **H** Lactate^{low}+TME^{low} subgroups. Each liver tumor from an individual patient was represented in each column (TCGA-KIRC). **I** Bar plot of *BAP1* gene mutation frequency differences between the Lactate^{high}+TME^{high} and Lactate^{low}+TME^{low} subgroups. **J** Kaplan–Meier overall survival curves of TCGA-KIRC patients (n=267) divided by *BAP1* mutation status and LAC-TME classifier

exhibited decreased gene expression with worsening prognosis, while Clusters 4 and 6 showed increased expression as prognosis deteriorated (Fig. 3A). KEGG and Reactome enrichment analyses revealed that Cluster 6 was predominantly associated with cell cycle processes. We then applied ssGSEA to score multiple datasets, including TCGA and E-MTAB-1980, and performed prognostic analysis. Patients with low ssGSEA scores for Clusters 3 and 5 had significantly better prognosis than those with high scores, whereas the opposite trend was observed for Clusters 4 and 6. These findings further validated the LAC-TME classifier's ability to stratify patient prognosis (Fig. 3B).

Enrichment analysis of the HALLMARK gene set revealed significant enrichment of pathways related to inflammation, cell cycle, IL6-JAK-STAT3 signaling, E2F targets, epithelial-mesenchymal transition (EMT), and G2M checkpoint in the Lactate^{high}+TME^{high} group. In contrast, the Lactate^{low}+TME^{low} group was enriched for mitochondrial-related metabolic pathways, including oxidative phosphorylation and fatty acid metabolism (Fig. 3C). Using the DecoupleR algorithm to infer pathway activity from transcriptomic data, we identified stark contrasts in signaling networks between the Lactate^{high}+TME^{high} and Lactate^{low}+TME^{low} subgroups. The Lactate^{low}+TME^{low} group exhibited elevated activity in pathways such as p53 and VEGF signaling, while the Lactate^{high}+TME^{high} group showed heightened activity in EGFR, PI3K, TRAIL, and NFκB pathways (Fig. 3D). GO-BP enrichment analysis indicated that the Lactate^{high}+TME^{high} group was primarily associated with cell cycle and immune-inflammatory processes, whereas the Lactate^{low}+TME^{low} group was linked to biological processes such as angiogenesis (Fig. 3E).

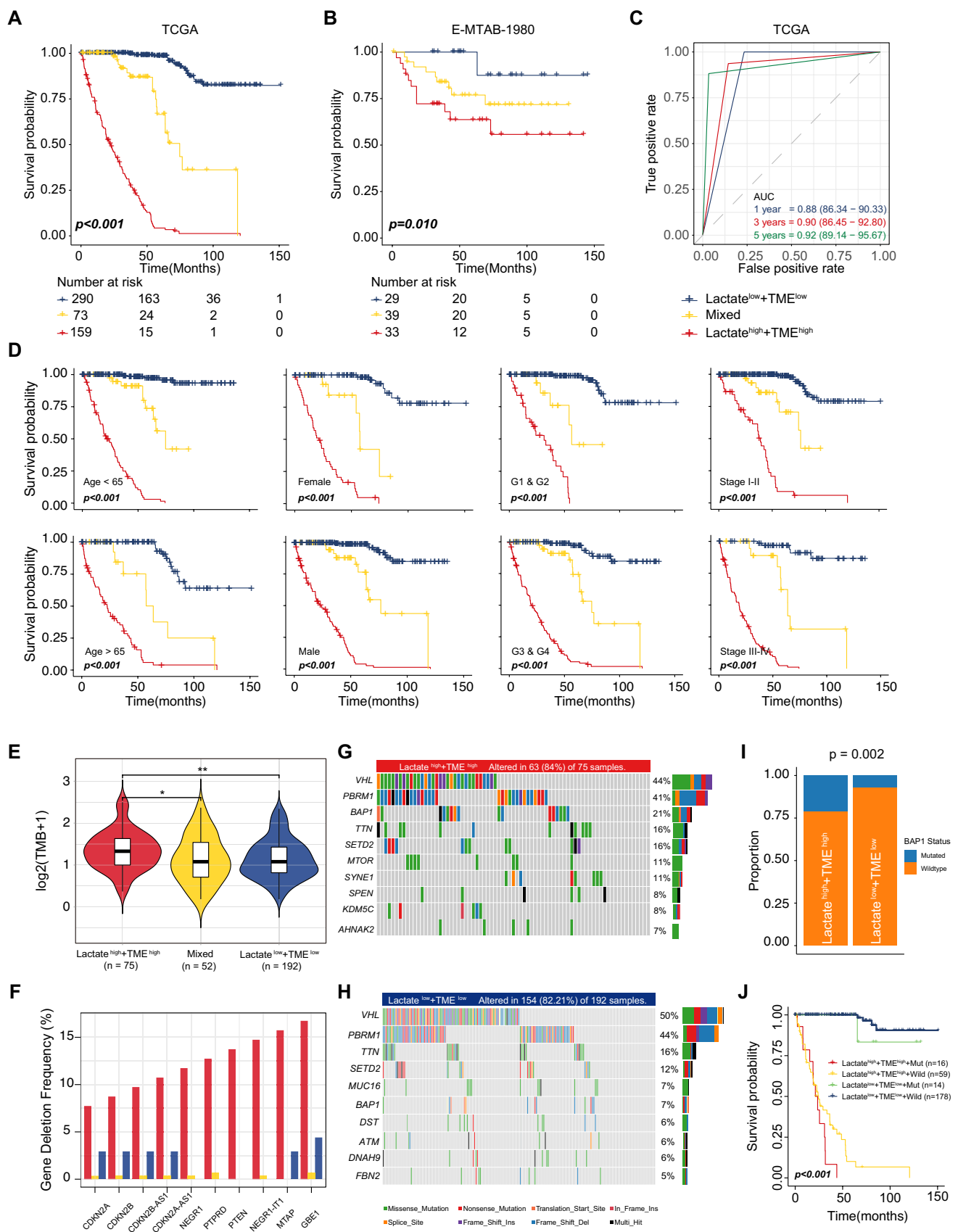
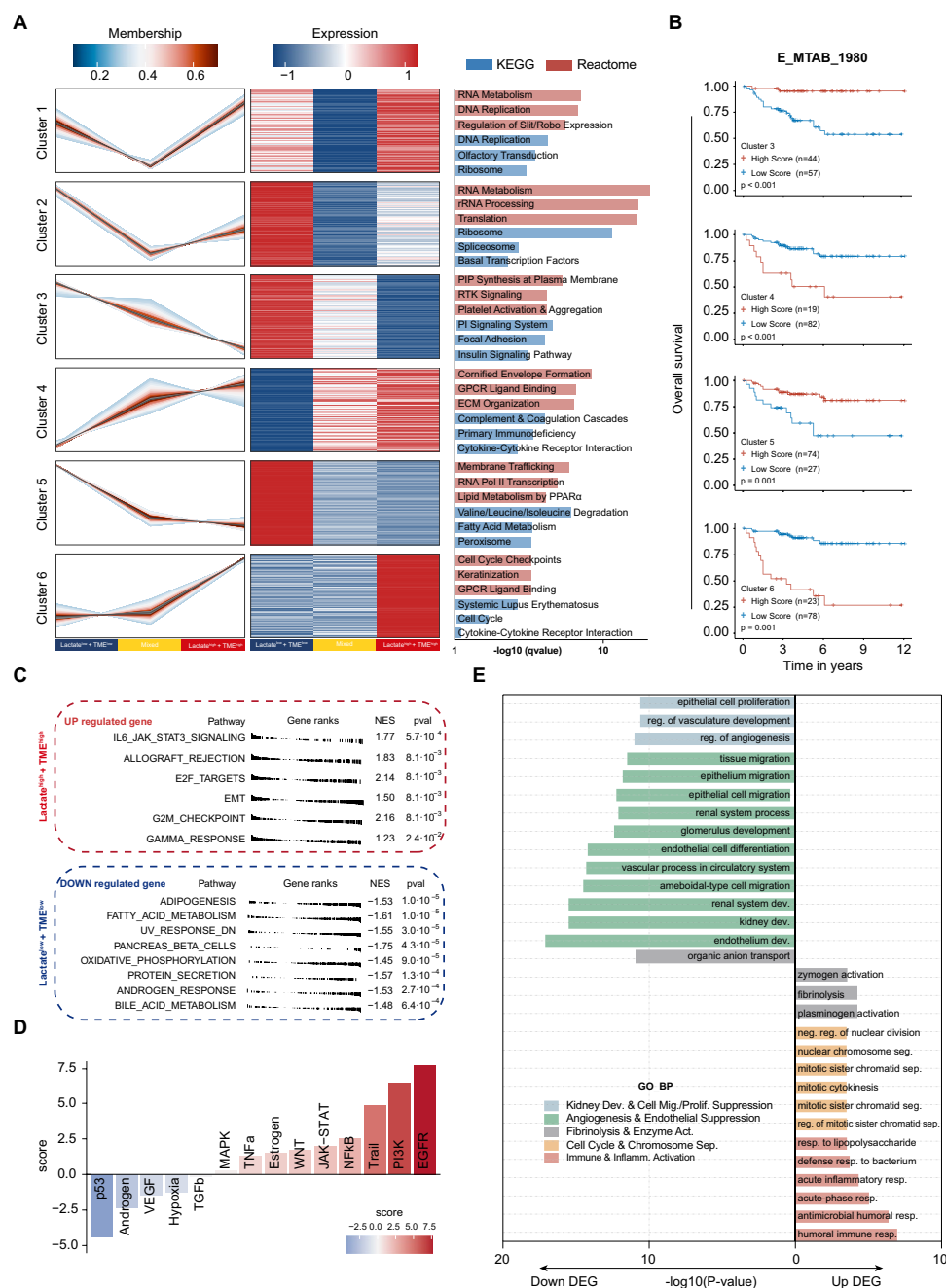


Fig. 3 Functional and Pathway Analysis of the LAC-TME Classifier: **A** Membership heatmap, gene expression heatmap, and KEGG/Reactome enrichment-analysis bar plots of 6 gene clusters from mFuzz analysis based on LAC-TME classifier prognosis. **B** Kaplan–Meier overall survival curves of ssGSEA scores for Cluster 3, 4, 5, and 6 genes in the E-MTAB-1980 cohort. **C** GSEA enrichment analysis results of HALLMARK gene sets. **D** Pathway activity scores based on DecoupleR algorithm analysis. **E**: GO_BP enrichment analysis plot

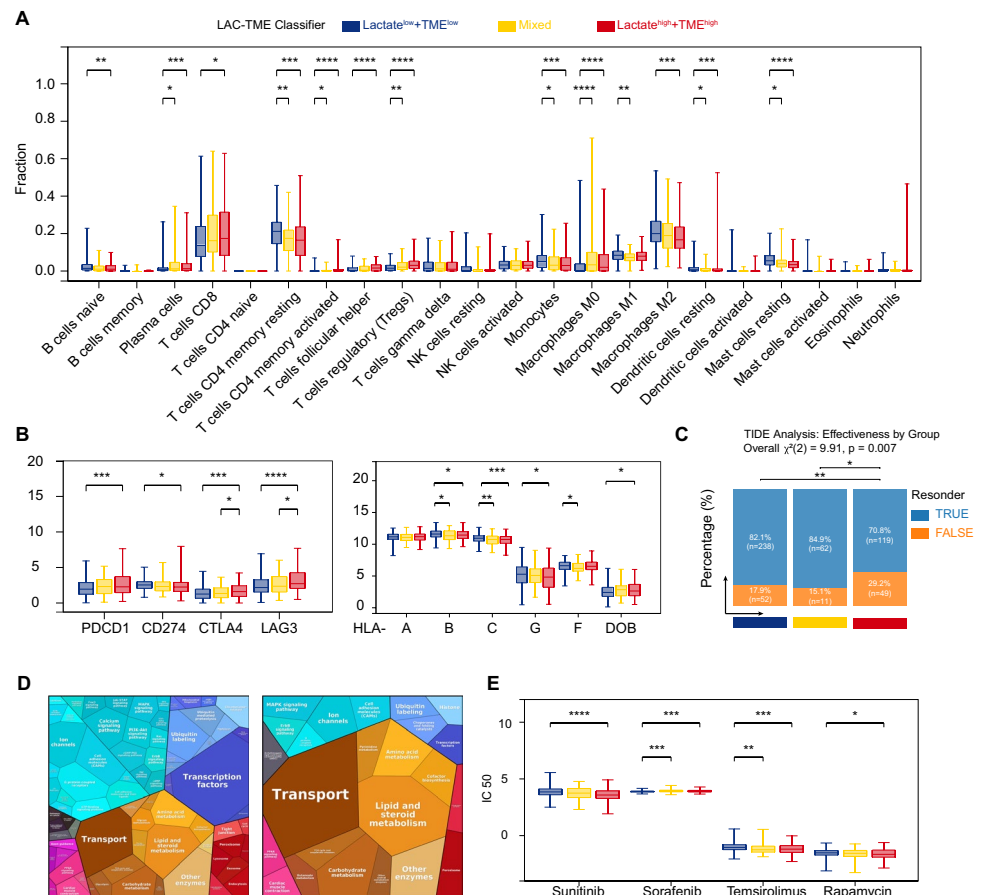


Immune microenvironment and treatment response analysis of the LAC-TME classifier

Systematic analysis of the immune microenvironment revealed marked heterogeneity between the Lactate^{low}+TME^{low} and Lactate^{high}+TME^{high} groups. CIBERSORT analysis indicated that the Lactate^{low}+TME^{low} group was enriched in resting immune cells, including naive B cells, resting CD4⁺memory T cells, monocytes, M2 macrophages, resting dendritic cells, and resting mast cells (Fig. 4A). In contrast, the Lactate^{high}+TME^{high} group was

characterized by activated CD8⁺T cells, regulatory T cells (Tregs), and plasma cells (all $p < 0.05$, Fig. 4A). Additionally, the Lactate^{high}+TME^{high} group exhibited significantly upregulated expression of immune checkpoint molecules such as *PDCD1*, *LAG3*, and *CTLA4* (Fig. 4B), but impaired antigen presentation capacity of HLA class I molecules (*HLA-B/C*). TIDE analysis further confirmed a significantly higher immunotherapy non-response rate (29.2% vs. 17.9%, $p < 0.01$, Fig. 4C). Proteomic profiling using Proteomap provided insights into the potential mechanisms underlying the LAC-TME classifier's ability to predict immunotherapy response (Fig. 4D). Notably, the proteomic

Fig. 4 Immune Microenvironment and Treatment Response Analysis of the LAC-TME Classifier: **A** Comparison of immune-cell differences among three LAC-TME subtypes by CIBERSORT analysis. **B** Comparison of the expression of immune-related genes in defined three subgroups based on LAC-TME classifier. Box and box plots showing normalized expression of mRNA for selected markers. **C** Boxplots depicting significantly upregulated expression of immune checkpoint molecules (*PDCD1*, *LAG3*, *CTLA4*) in the Lactate-high + TME-high group. **C** TIDE analysis bar plot showing immune therapy response rates of three LAC-TME subtypes. **D** Functional analysis in Lactate-low + TME-high (left) and Functional analysis in responder of patients under TIDE analysis illustrated using Proteomaps. **E** Box plots of drug sensitivity analysis of the LAC-TME classifier to sunitinib, temsirolimus, rapamycin, and sorafenib



profile of the Lactate^{low}+TME^{low} group closely resembled that of TIDE responders. Collectively, these findings suggest that baseline LAC-TME features may delineate the tumor immune microenvironment, aiding in the prediction of treatment response.

The LAC-TME classification significantly influenced sensitivity to targeted therapies in ccRCC (Fig. 4E). The Lactate^{low}+TME^{low} group exhibited significant resistance to sunitinib ($p < 0.001$), temsirolimus ($p < 0.001$), and rapamycin ($p < 0.05$), potentially due to oxidative phosphorylation-driven energy metabolism. Conversely, the Lactate^{high}+TME^{high} group showed reduced sensitivity to sorafenib ($p < 0.001$), likely attributable to active EGFR/PI3K bypass signaling.

Prognostic independence and nomogram development for the LAC-TME classifier

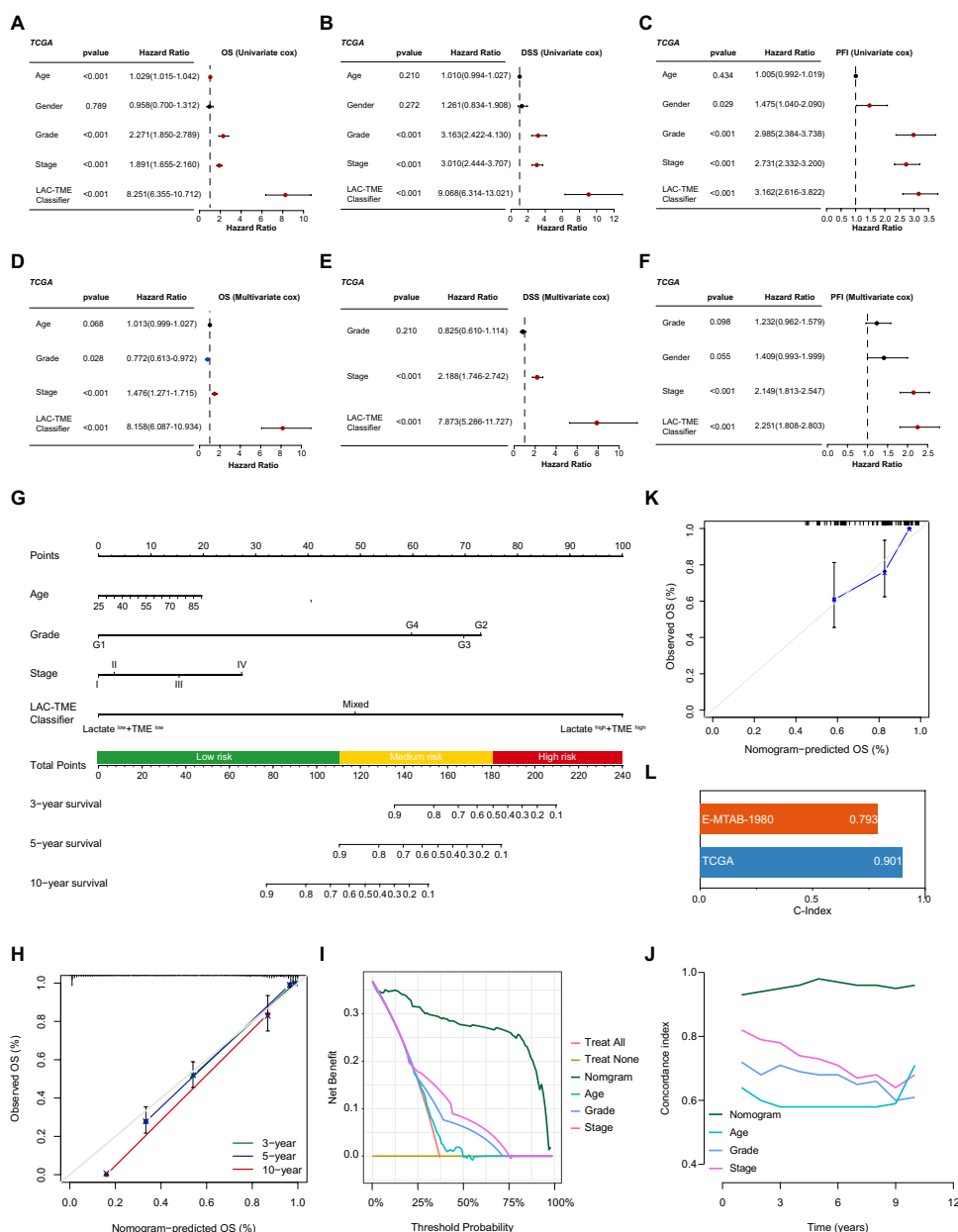
Univariate and multivariate Cox regression analyses established the LAC-TME classifier as an independent prognostic factor for ccRCC (Fig. 5A-F). In OS analysis, the multivariate hazard ratio (HR) for the LAC-TME classifier was 8.158 ($p < 0.001$), surpassing tumor stage (HR = 1.576) and grade (HR = 0.772). For disease-specific survival (DSS),

the LAC-TME classifier consistently exhibited the highest HR ($p < 0.001$). Based on these analyses, we integrated key prognostic factors to develop a nomogram model for ccRCC. The nomogram incorporated the LAC-TME classifier, tumor stage, and grade, enabling quantitative prediction of 3-, 5-, and 10-year survival probabilities (Fig. 5G) and showed good calibration (Fig. 5H). The time-dependent C-index reached 0.95, and decision curve analysis (DCA) demonstrated significantly greater clinical net benefit compared to individual predictors (Fig. 5I, J). In the external cohort (E-MTAB-1980), the C-index was 0.793, with well-fitted calibration curves (Fig. 5K, L), indicating robust predictive performance across cohorts.

Expression and functional role of *LGALS1* in clear cell renal cell carcinoma

Based on previous analyses, we selected *LGALS1* as the core molecule for further investigation due to its significantly differential expression and high feature importance in the model (Fig. 6A). In the TCGA database, patients with low *LGALS1* expression exhibited remarkably longer OS (Fig. 6B) and progression-free survival (PFS, Fig. 6C) than those with high expression. This prognostic trend was

Fig. 5 Prognostic Independence and Nomogram Development for the LAC-TME Classifier: **A-C** Univariate regression analysis of the LAC-TME classifier, age, sex, tumor grade, and tumor stage for OS, DSS, and Progression Free Interval (PFI). **D-F** Multivariate regression analysis of the LAC-TME classifier, age, sex, tumor grade, and tumor stage for OS, DSS, and PFI. **G** Prognostic nomogram for 3-, 5-, and 10-year survival probabilities based on the LAC-TME classifier, age, sex, tumor grade, and tumor stage. **H** Calibration curves for 3-, 5-, and 10-year survival predictions of the nomogram. **I** Decision curve analysis of the nomogram and individual predictive variables. **J** Time-dependent C-index curves of the nomogram and individual predictive variables. **K** Calibration curves for external validation of the nomogram in the E-MTAB-1980 cohort. **L** Histogram of the overall C-index of the nomogram in the TCGA and E-MTAB-1980 cohorts

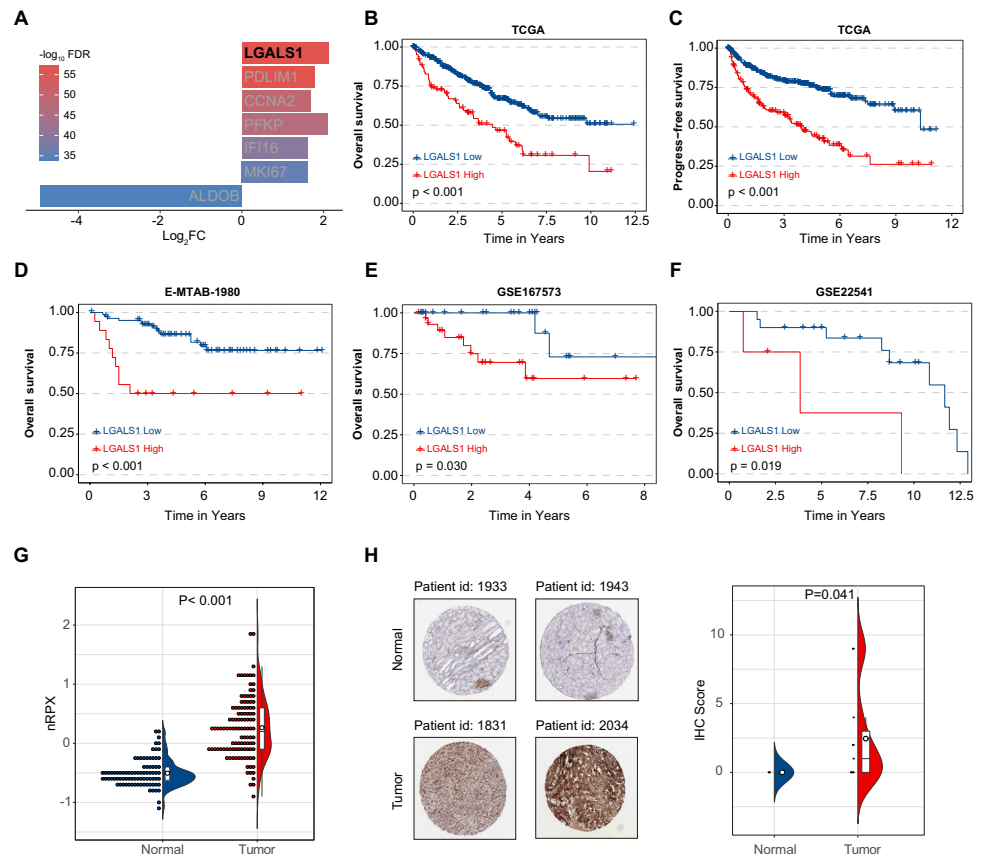


consistently validated across multiple independent cohorts, including E-MTAB-1980, GSE167573, and GSE22541, where low *LGALS1* expression was linked to better survival outcomes (Fig. 6D-F). Furthermore, analysis of the CPTAC mass spectrometry data from the Human Protein Atlas revealed that *LGALS1* expression was substantially higher in ccRCC tissues than in normal tissues (Fig. 6G). Immunohistochemical staining also confirmed that *LGALS1* was markedly upregulated in ccRCC tissues compared to normal controls (Fig. 6H).

To explore the functional role of *LGALS1*, we conducted siRNA-mediated *LGALS1* knockdown experiments in the 786-O and 769-P renal cancer cell lines. qPCR results showed that all three siRNAs significantly reduced *LGALS1*

mRNA levels in both cell lines (Fig. 7A). Western Blot confirmed the corresponding decrease in *LGALS1* protein expression (Fig. 7B). CCK8 proliferation assays indicated that *LGALS1* knockdown significantly suppressed the proliferative capacity of 786-O and 769-P cells compared to the negative control (NC) group (Fig. 7C). Transwell migration assays demonstrated that *LGALS1* knockdown significantly inhibited the migratory ability of renal cancer cells (Fig. 7D). Similarly, Transwell invasion assays showed a marked reduction in the invasive capacity of these cells following *LGALS1* knockdown (Fig. 7E). Wound healing assays further confirmed that *LGALS1* knockdown inhibited the migratory ability of 786-O (Fig. 7F) and 769-P (Fig. 7G) cells. Flow cytometric analysis of the cell cycle revealed

Fig. 6 *LGALS1* Expression and Prognostic Analysis **A** Histogram showing the expression of the optimal prognostic characterization of lactate-related genes. **B** Kaplan–Meier overall survival curves of TCGA-KIRC patients divided by *LGALS1* expression. **C** Kaplan–Meier progress free survival curves of TCGA-KIRC patients divided by *LGALS1* expression. **D** Kaplan–Meier overall survival curves of E-MTAB-1980, **E** GSE167573, **F** GSE22541 cohorts divided by *LGALS1* expression. **G** Differential expression of *LGALS1* protein in ccRCC and normal tissues from CPTAC mass spectrometry data in the Human Protein Atlas. **H** Immunohistochemistry (IHC) images of *LGALS1* in normal kidney and ccRCC tissues from the Human Protein Atlas (Antibody CAB002157, left). Comparative plot of IHC scores for *LGALS1* between the two groups (right)



that *LGALS1* knockdown led to a significant increase in the proportion of 786-O cells in the G0/G1 phase, along with a reduction in the S and G2/M phases (Fig. 7H).

In summary, these findings suggest that *LGALS1* plays a crucial role in the development and progression of ccRCC by promoting cell proliferation, migration, invasion, and cell cycle progression. Thus, *LGALS1* may represent a potential therapeutic target for ccRCC.

Discussion

As a highly heterogeneous tumor driven by metabolic abnormalities, ccRCC urgently requires breakthroughs in precise molecular classification systems to improve prognosis assessment and treatment decisions. This study introduces the LAC-TME classifier, integrating lactate metabolism and TME interactions via machine learning, surpassing traditional single-omics approaches by capturing complex metabolism-immune dynamics.

Compared to prior ccRCC molecular subtyping efforts, such as those by TCGA (Beuselinck et al. 2015; Cancer Genome Atlas Research Network 2013), which characterized metabolic and immune landscapes separately, the LAC-TME classifier uniquely integrates lactate-related

genes and TME features using machine learning, offering superior prognostic stratification (C-index 0.92) and actionable therapeutic insights, particularly for prioritizing immunotherapy or targeted therapies like Sunitinib in the Lactate^{high}+TME^{high} subgroup.

Elevated lactate levels not only provide energy support for tumor cells (Chen et al. 2025) but may also suppress immune cell function by acidifying the microenvironment (Watson et al. 2021; Certo et al. 2021) (Peralta et al. 2024; Cheng et al. 2023), thereby promoting immune evasion. Mfuzz clustering analysis further elucidates dynamic gene expression patterns: as prognosis worsens, expression levels in Clusters 4 and 6 (associated with cell cycle and inflammatory pathways) increase, while those in Clusters 3 and 5 (related to metabolic homeostasis) decrease. ssGSEA analysis confirms that the pathway activities of these clusters are highly correlated with survival outcomes, reinforcing the critical role of lactate metabolism in ccRCC progression. HALLMARK enrichment analysis shows that the Lactate^{high}+TME^{high} group is enriched in cell cycle and inflammation-related pathways, while the Lactate^{low}+TME^{low} group is enriched in mitochondrial metabolic pathways, further explaining the molecular basis of prognostic differences.

In the Lactate^{high}+TME^{high} group, immune checkpoint molecules are significantly upregulated, but the antigen

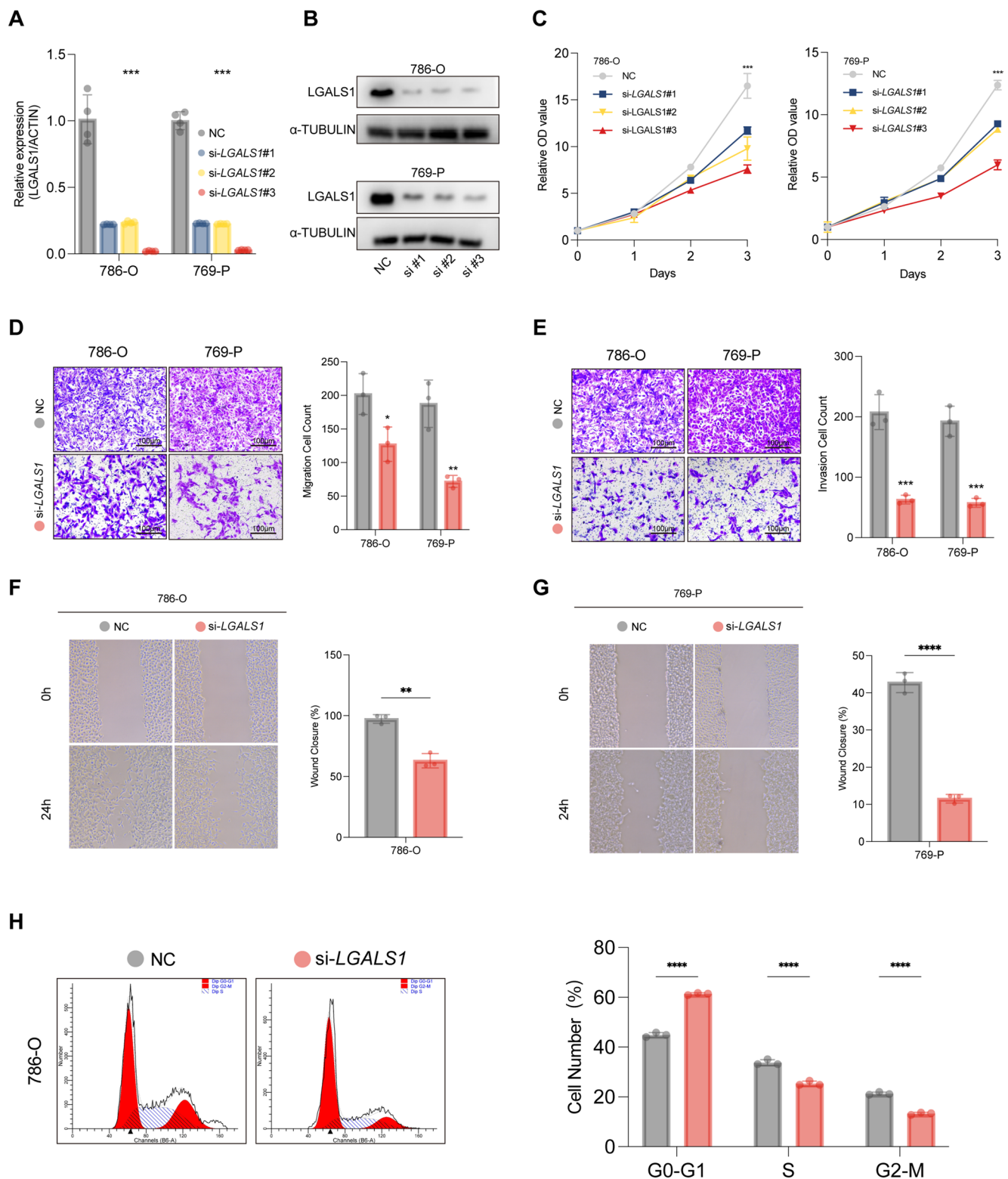


Fig. 7 Functional Validation of LGALS1 in ccRCC **A** qPCR validation of mRNA knockdown efficiency in 786-O and 769-P cell lines after *LGALS1* interference. **B** Western Blot validation of protein knockdown efficiency in 786-O and 769-P cell lines after *LGALS1* interference. **C** CCK-8 assay for cell proliferation curves in 786-O and 769-P cell lines after *LGALS1* interference. **D** Transwell assay for cell migration in 786-O and 769-P cell lines after *LGALS1* interference. **E**.

Transwell assay for cell invasion in 786-O and 769-P cell lines after *LGALS1* interference. **F** Wound healing assay for cell migration in 786-O cell line after *LGALS1* interference. **G** Wound healing assay for cell migration in 769-P cell line after *LGALS1* interference. **H** Flow cytometry analysis of cell cycle distribution in 786-O cell line after *LGALS1* interference.

presentation capacity of HLA class I molecules is impaired, potentially leading to T cell dysfunction (Hazini et al. 2021). CIBERSORT analysis indicates that the Lactate^{high}+TME^{high} group is enriched in activated CD8⁺ T cells and regulatory T cells (Tregs), constituting an immunosuppressive microenvironment (Zhou et al. 2024) (Shan et al. 2022; Kurebayashi et al. 2021), whereas the Lactate^{low}+TME^{low} group is dominated by resting immune cells. TIDE analysis further demonstrates that the immunotherapy non-response rate in the Lactate^{high}+TME^{high} group is significantly higher than in other groups, with a high Dysfunction score reflecting severe T cell exhaustion. Additionally, proteomic profiling (Proteomap) shows that the Lactate^{low}+TME^{low} group shares similar features with immunotherapy responders, highlighting the potential value of this classification system in predicting immunotherapy response.

Drug sensitivity analysis reveals that the Lactate^{high}+TME^{high} group exhibits higher sensitivity to targeted therapies such as Sunitinib, Temsirolimus, and Rapamycin, but reduced sensitivity to Sorafenib due to enhanced EGFR/PI3K pathway activity (Dong et al. 2017; Zhang et al. 2018; Chen et al. 2011) (Ezzoukhry et al. 2012; He et al. 2021). These results suggest that the LAC-TME classification can guide the selection of targeted therapies; for instance, PI3K/mTOR inhibitors may be prioritized for Lactate^{high}+TME^{high} patients, while the Lactate^{low}+TME^{low} group, with its lower immunosuppressive features, may be more suitable for immune checkpoint inhibitors.

Furthermore, our study identified *LGALS1* as a key molecule within the LAC-TME classifier, exhibiting significant differential expression and high feature importance. High *LGALS1* expression was associated with poorer OS and PFS in multiple cohorts. Cell functional experiments demonstrated significant impact in cell proliferation, migration, and invasion, alongside cell cycle arrest in the G0/G1 phase. These findings underscore *LGALS1*'s pivotal role in ccRCC progression and its potential as a therapeutic target, further validating the biological relevance of the genes included in the LAC-TME classifier.

The LAC-TME classifier shows strong potential but has limitations. First, its retrospective data need prospective validation. Future multicenter studies, including ICI-treated ccRCC cohorts, will compare it with existing predictors to confirm its prognostic and predictive value. Second, the interplay between lactate metabolism and TME, such as *BAP1* mutation effects, requires further study. The model's robust performance in training and validation cohorts supports its clinical utility, with plans for additional analyses like feature stability to enhance it. Lastly, clinical use requires standardized testing and multicenter trial support.

Conclusion

In conclusion, this study establishes the LAC-TME classification system as a powerful tool for elucidating the critical roles of lactate metabolism and the TME in ccRCC prognosis and treatment response. The system not only clarifies the biological mechanisms of metabolic-immune interactions at the molecular level but also provides a practical methodological framework for precise classification and personalized treatment. Future research should delve deeper into the mechanisms underlying lactate metabolism and TME interactions and validate the clinical utility of this classification system in multicenter, large-scale trials to facilitate its translation into clinical practice.

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Data availability The data presented in the study are included in the article and further data are shared with researchers who provided an approved proposal. Requests for data should be directed by email to Yinzhaoh Wang. Data requestors will need to sign a data access agreement.

Declarations

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

Ethical approval This study utilized publicly available datasets from The Cancer Genome Atlas (TCGA), E-MTAB-1980, and The Human Protein Atlas. As these datasets are publicly accessible and do not contain any personally identifiable information, ethics approval was not required. The study adhered to all relevant guidelines for the use of publicly available data.

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