

A five-gene prognostic signature integrating endothelial senescence and lipid metabolism for risk stratification in hepatocellular carcinoma

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1 **A five-gene prognostic signature integrating endothelial senescence and lipid**
2 **metabolism for risk stratification in hepatocellular carcinoma**

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3

Abstract

4 **Background:** Hepatocellular carcinoma (HCC) is closely associated with endothelial
5 cell senescence and lipid metabolism (EL), yet shared mechanisms remain unclear.
6 Hence, elucidating the mechanisms linking HCC to EL-related genes (ELRGs) is
7 crucial for timely diagnosis and effective management of HCC.

8 **Methods:** Sequencing datasets related to HCC were obtained from publicly available
9 databases. By intersecting differentially expressed genes (DEGs), endothelial cell
10 senescence-related genes (ESRGs), and lipid metabolism-related genes (LMRGs),
11 candidate genes were obtained. Prognostic genes were obtained through univariate
12 Cox regression analysis and machine learning. Next, HCC samples were divided into
13 two risk groups. Subsequent analyses focused on functional enrichment and others.
14 Single-cell RNA sequencing (scRNA-seq) analysis was further performed to identify
15 the primary cellular sources of the prognostic genes, characterize their expression
16 dynamics along developmental trajectories, and reconstruct intercellular
17 communication networks across distinct cell types. Lastly, the expression of
18 prognostic genes in tumor tissue samples was verified by reverse transcription
19 quantitative polymerase chain reaction (RT-qPCR).

20 **Results:** A total of 52 candidate genes were identified. Subsequently, five prognostic
21 genes were identified and used to establish a prognostic model. Analysis illustrated
22 that two risk groups were co-enriched in pathways related to metabolic pathways. The
23 scRNA-seq analysis demonstrated hepatocyte-predominant expression of all five

1 prognostic genes, with tumor-specific upregulation and stage-specific pseudotime
2 dynamics. Cell-cell communication analysis further identified the NAMPT–
3 (ITGA5+ITGB1) signaling axis as a key mediator of intercellular crosstalk. RT-qPCR
4 analysis showed significant upregulation of FABP5, FABP6 and UGT1A10, but
5 downregulation of MMP1 and SGPP2 in HCC samples.

6 **Conclusions:** A five-gene prognostic signature (FABP5, FABP6, MMP1, SGPP2,
7 UGT1A10) was established and validated. Among these, FABP5 showed the strongest
8 correlation with activated CD4⁺ T-cell infiltration (cor = 0.52, P < 0.05) and was
9 predominantly expressed in hepatocytes with stage-specific upregulation along
10 pseudotime trajectories. Single-cell analysis further revealed NAMPT–
11 (ITGA5+ITGB1) as a key intercellular signaling axis. These findings suggest that
12 FABP5 may serve as a link between endothelial senescence, lipid metabolism, and
13 immune microenvironment remodeling in HCC, warranting further functional
14 investigation.

15

16 **Keywords:** Hepatocellular carcinoma, Endothelial cell senescence, Lipid metabolism,
17 Prognostic genes

18

19 **Background**

20 Ranked as the sixth most prevalent cancer worldwide, primary liver cancer is also the
21 third leading cause of cancer-related deaths[1], with hepatocellular carcinoma (HCC)
22 being the most common form, representing about 75-85% of cases[2]. Studies have

1 demonstrated that hepatitis B and C virus (HBV and HCV) infections, aflatoxin
2 exposure, polluted drinking water, nonalcoholic fatty liver disease, overweight, type 2
3 diabetes, heavy alcohol consumption, and cigarette smoking are associated with
4 HCC[2, 3]. In recent years, the primary risk factors for HCC have changed
5 significantly, with hepatitis B virus and hepatitis C virus infections decreasing in
6 many places, while in parallel, overweight and type 2 diabetes mellitus (T2DM) have
7 increased in prevalence and have become increasingly important risk factors in the
8 development of HCC[4]. Currently, treatments for HCC include surgery,
9 chemotherapy, radiotherapy, liver transplantation, targeted therapy, immunotherapy,
10 and traditional Chinese medicine[1, 5]. Despite these treatments, patients with HCC
11 may experience recurrence, may metastasize to other tissues or organs even after
12 treatment, and the prognosis for advanced HCC remains poor[5]. The high prevalence
13 and low survival rates of HCC are mainly due to the heterogeneity of the causative
14 factors, therapeutic responses, and molecular profiles[6]. Therefore, validation of new
15 and reliable HCC biomarkers may help to identify new targets for disease diagnosis or
16 prognosis prediction and improve the accuracy of prognosis prediction.

17 Cellular senescence is a defense mechanism that can be induced by a variety of
18 endogenous or exogenous stimuli as well as injury signals, and it has been found that
19 Cellular senescence exerts context-dependent effects: tumor-cell-intrinsic senescence
20 can restrain proliferation, whereas senescent endothelial cells may remodel the tumor
21 microenvironment through SASP-mediated inflammatory and immunoregulatory
22 signals[7, 8], and inhibition of HCC cellular senescence promotes HCC cell

1 proliferation[9], and promotion or induction of cellular senescence inhibits the
2 proliferation of HCC cells[10, 11], and endothelial cells play a critical role in tumor
3 microenvironment, which significantly affects tumor development and
4 progression[12]. Senescent endothelial cells can promote tumor growth and
5 dissemination[13, 14], and research has indicated that factors released by senescent
6 endothelial cells as part of the senescence-associated secretory phenotype (SASP)
7 have been demonstrated in uveal melanomas to promote the proliferation and invasion
8 of tumor cells and to affect the immune cells in the tumor microenvironment[13]. In
9 addition, the rate of glycolysis in endothelial cells further increases during aging and
10 is linked to several age-related diseases (ARD), such as atherosclerosis, diabetes, and
11 cardiovascular diseases[15].

12 Lipid metabolism (LM) is an important and complex biochemical reaction in vivo,
13 which plays a role in controlling cellular activities like cell growth, differentiation,
14 and programmed cell death[16]. Numerous studies have shown that abnormalities in
15 LM are significantly linked to HCC development[17-20], such as MiR-612 regulates
16 the invasiveness of HCC through HADHA-mediated lipid reprogramming[21]. In
17 addition, it was found that disorders of fatty acid metabolism promote vascular
18 endothelial cell senescence (ES) through acetyl coenzyme a-induced protein
19 acetylation modification[22], suggesting a potential link between ES and LM. Given
20 the relationship between ES and LM and their respective roles in HCC, Lipid
21 metabolic reprogramming in HCC may influence not only tumor-cell bioenergetics
22 but also vascular and immune remodeling through altered lipid handling, redox

balance, and immunometabolic signaling[22]. screening for biomarkers associated with endothelial cell senescence and LM in HCC is important to identify new potential targets for HCC therapy and prevention.

In this study, the predictive significance of genes linked to ES and LM in HCC was characterized using bioinformatics methods based on transcriptomics data from public databases. On this basis, the roles and functions of the screened prognostic genes in HCC were further explored. Further validation was performed using reverse transcription quantitative polymerase chain reaction (RT-qPCR) of clinical samples, aiming to provide novel ideas for early prevention of HCC patients and clinical drug development.

Methods

Data source

The TCGA-LIHC training set, including 368 HCC samples and 50 adjacent normal tissues, was retrieved from The Cancer Genome Atlas (TCGA) on June 26, 2024. The GSE15654 (GPL8432) dataset was obtained from the Gene Expression Omnibus (GEO) as a validation set, which contains 216 HCC tissue cohorts. Additionally, scRNA-seq data (GSE149614 and GPL24676 platforms) were retrieved from the GEO database on March 6, 2025. These data were derived from samples of ten HCC patients and five adjacent normal tissues. Moreover, 102 endothelial cell senescence-related genes (ESRGs) were collected from the literature[14]. The 1,045 lipid metabolism-related genes (LMRGs) were also gathered from existing

1 literature[23].

2 **Differential gene expression analysis**

3 The comparison of differentially expressed genes (DEGs) within TCGA-LIHC dataset
4 was conducted between HCC and control groups, employing DESeq2 (v 1.34.0)
5 package[24] (adjusted $P < 0.05$, $|\log_2 \text{FoldChange (FC)}| > 0.5$). The result was
6 visualized by a volcano map via ggplot2 (v 3.4.1) package[25] and a heatmap via
7 ComplexHeatmap (v 2.14.0) package[26].

8 **Weighted gene co-expression network analysis (WGCNA)**

9 Research delved into the identification of modules of strongly correlated genes,
10 further exploring the intricate connections between the modules and the association
11 with phenotypic traits utilizing WGCNA. Subsequently, univariate Cox regression
12 was performed via survival (v 3.5-3) package[27] (hazard ratio (HR) $\neq 1$, $P < 0.05$),
13 specifically targeting the expression of 102 ESRGs. Utilizing GSVA (v 1.42.0)
14 package[28], the investigators calculated the expression scores of ESRGs in HCC
15 samples. Following this, HCC samples were stratified into two score groups via the
16 most suitable cut-off points. Furthermore, survminer (v 0.4.9) package[29] was
17 employed to generate Kaplan-Meier (KM) survival curves for both groups.

18 Next, WGCNA (v 1.70.3) package[30] was used to identify key module genes.
19 Initially, a clustering analysis was performed across HCC samples to determine
20 whether any outliers needed to be excluded. The optimal soft threshold (β) was
21 established by targeting $R^2 = 0.85$. The scale-free network was constructed utilizing β ,
22 which enabled the division of genes into distinct modules. Subsequently, the scores of
23 ESRGs previously derived were employed as phenotypic markers to scrutinize the

1 relationship between these phenotypes and the identified modules, with the aim of
2 pinpointing key module genes associated with endothelial cell senescence prognosis.

3 **Functional enrichment**

4 The candidate genes were identified by intersecting DEGs, key module genes and
5 1,045 LMRGs, utilizing VennDiagram (v 1.7.1) package[31]. Furthermore, functional
6 enrichment analyses of candidate genes were conducted through Gene Ontology (GO)
7 and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways via clusterProfiler
8 (v 4.2.2) package[32] ($P < 0.05$). The researchers employed the STRING database to
9 construct a protein-protein interaction (PPI) network (confidence level > 0.4).

10 **A prognostic risk model was constructed and rigorously validated**

11 Utilizing the TCGA-HCC, survival-related candidate genes were identified through a
12 combined approach of univariate Cox regression analysis, leveraging survival (v 3.5-3)
13 package[27]. The glmnet (v 4.1-4) package was used to perform the Least absolute
14 shrinkage and selection operator (LASSO)[33]. Specifically, LASSO analysis
15 incorporated a 10-fold cross-validation strategy, and further obtained prognostic genes.
16 The research undertook a comprehensive evaluation of the predictive value of
17 prognostic risk models. The median risk score was calculated by using the cutoff
18 function, which divided HCC patients into a high-risk group (HRG) and a low-risk
19 group (LRG). The risk score calculation incorporated the risk coefficient (coef) and
20 expression level (expr) of each gene:

21
$$Risk\ score = \sum_{i=1}^n coef(gene_i) * expr(gene_i)$$

22 Next, KM survival curves were constructed via the survminer (v 0.4.9) package.

23 Receiver operating characteristic (ROC) curves were plotted through the survivalROC

1 (v 1.0.3) package[34], and predictive performance was quantified by calculating the
2 area under the curve (AUC) value. Moreover, the constructed model was validated in
3 the same way based on GSE15654.

4 To further investigate the independent prognostic model, the clinicopathologic
5 characteristics encompassed age, gender, race, and T stage, within TCGA-HCC
6 dataset. Additionally, a univariate Cox regression analysis incorporated the risk score
7 obtained from the risk model. The proportional hazard (PH) assumption test was
8 performed and subsequently incorporated into a multivariate Cox regression analysis.
9 The TCGA-HCC dataset was utilized to incorporate the identified independent
10 prognostic factors into a prognostic nomogram via rms (v 6.5-1) package[35].
11 Calibration and ROC curves were generated to assess the predictive accuracy of
12 nomogram. Furthermore, disparities in risk scores across various clinical subgroups in
13 TCGA-HCC cohort were examined via Wilcoxon test ($P < 0.05$).

14 **Gene Set Enrichment Analysis (GSEA)**

15 DESeq2 package was employed for conducting the difference analysis within HRG
16 and LRG, and $\log_2FC$ values were computed and ranked from the most significant to
17 the least. Signaling pathways were identified via GSEA enrichment analysis with
18 Molecular Signatures Database (MSigDB, 2.cp.kegg.v2022.1.Hs.symbols.gmt) (False
19 Discovery Rate (FDR) < 0.05 , $|\text{Normalized enrichment score (NES)}| > 1$). The
20 outcomes of this analysis were then displayed via clusterProfiler (v 4.2.2)
21 package[32].

22 **Immune analysis**

23 To comprehensively investigate immune cell infiltration, researchers employed GSVA
24 (v 1.42.0) package to implement single sample gene set enrichment analysis (ssGSEA)
25 method, profiling 28 types of immune cells[36]. Subsequently, the heatmap and the

1 box plot were plotted based on immune infiltrating cell enrichment scores, comparing
2 those between HRG and LRG ($P < 0.05$). Spearman correlation analysis was then
3 applied to explore associations between prognostic genes and immune cell
4 populations, utilizing psych package[37]. Finally, a correlation heatmap was
5 generated and visualized with corrplot (v 0.92) package[38].

6 Utilizing Wilcoxon test, the expression profiles of 48 immune checkpoint molecules
7 were compared between HRG and LRG within TCGA-HCC dataset. The ESTIMATE
8 algorithm was applied to assess the stromal, immune, and overall ESTIMATE scores
9 of HCC samples sourced from TCGA-HCC, utilizing the estimate package (v
10 1.0.13)[39].

11 Utilizing tumor immune dysfunction and exclusion (TIDE), the researchers procured
12 the scores of TIDE, Dysfunction and Exclusion for the samples, which were
13 subsequently subjected to analysis via Wilcoxon test.

14 Within TCGA-HCC, somatic mutation data were obtained for each tumor sample, to
15 identify the most frequently mutated genes in HRG and LRG, maftools (v 2.14.0)
16 package[40] was employed for comprehensive analysis. A tumor mutation burden
17 (TMB) model was constructed by dividing HCC patients with survival data into high
18 and low TMB groups based on the median TMB value derived from TCGA-HCC
19 cohort. Next, survival analysis was performed utilizing via survival package, and KM
20 survival curves were plotted using the survminer (v 0.4.9) package.

21 Besides, through the utilization of cBioPortal, the study retrieved the mutation
22 blueprints of each prognostic gene.

23 **Analysis of drug sensitivity**

24 Employing pRRophetic (v 0.5) package[41], the study estimated the half-maximal
25 inhibitory concentration (IC_{50}) of patients to various common chemotherapeutic drugs

1 to assess the predictive power of the drugs. Subsequently, statistical significance in
2 IC_{50} of the drugs between HRG and LRG of HCC was utilized Wilcoxon test ($P <$
3 0.05).

4 Protein expression alterations of each prognostic gene in HCC were retrieved via the
5 Human Protein Atlas (HPA), a large public database where protein expression profiles
6 of normal human tissues, organelles, blood cells and cancer tissues were provided.

7 **Construction of mRNA-miRNA-lncRNA interaction**

8 The prognostic genes associated microRNAs (miRNAs) were anticipated through
9 miRNet database as well as NetworkAnalyst database, and intersections were then
10 taken. Similarly, long noncoding RNAs (lncRNAs) were anticipated through the
11 miRNet database and StarBase database, and intersections were then taken.
12 Additionally, transcription factors (TFs) were anticipated through miRNet database.
13 Finally, an integrated regulatory network encompassing mRNA-miRNA-lncRNA
14 interactions, along with a TF-mRNA network, was constructed via Cytoscape
15 software (v 3.8.2) [42].

16 **Preprocessing of scRNA-seq data and cell type annotation**

17 To ensure data quality for downstream analysis, stringent quality control and
18 preprocessing were performed on the scRNA-seq data using the Seurat package (v
19 5.0.1)[43]. The following filtering criteria were applied to retain high-quality cells: (i)
20 genes detected in fewer than three cells were excluded; (ii) cells with fewer than 200
21 or more than 6,000 detected genes were removed; (iii) cells in which the
22 mitochondrial gene fraction exceeded 10% were excluded; and (iv) cells with a total
23 unique molecular identifier (UMI) count exceeding 20,000 were removed. Following

1 quality control, data normalization was performed using the NormalizeData function
2 with default parameters. Subsequently, the top 2,000 highly variable genes (HVGs)
3 were identified using the FindVariableFeatures function with the variance-stabilizing
4 transformation (vst) method. The data were then scaled using the ScaleData function,
5 followed by principal component analysis (PCA) for dimensionality reduction. The
6 number of significant PCs was determined based on an elbow plot, and the selected
7 PCs were subsequently used for graph-based clustering using the FindNeighbors and
8 FindClusters functions, with the resolution parameter set to 0.1. For visualization of
9 the high-dimensional data, uniform manifold approximation and projection (UMAP)
10 was employed. Cluster-specific marker genes were identified using the
11 FindAllMarkers function. Cell-type annotation was subsequently performed by
12 comparing the identified marker genes against established cell-type signatures
13 reported in the literature[44].

14 **Single-cell exploration of expression features, pseudotime trajectories and**
15 **intercellular communication of prognostic genes**

16 To identify the primary cellular sources of the prognostic genes, their expression
17 distribution across the annotated cell types was examined. The expression levels of
18 prognostic genes were compared between tumor and control samples within each cell
19 type using the Wilcoxon test, with a significance threshold set at $P < 0.05$. Cell types
20 exhibiting significant differential expression of these prognostic genes between the
21 two groups were selected for downstream analyses. Subsequently, dimensionality
22 reduction and re-clustering were performed on these selected cell types at a resolution

1 parameter of 0.1. To further elucidate the cell-state trajectories of these cells and
2 characterize the temporal expression dynamics of the prognostic genes during cellular
3 state transitions, pseudotime trajectory analysis was conducted using the Monocle2
4 algorithm (v 2.26.0)[45]. Briefly, cells were ordered along a learned trajectory based
5 on their transcriptomic profiles. The expression patterns of the prognostic genes along
6 the pseudotime axis were subsequently visualized to explore their potential roles
7 during cellular differentiation. Furthermore, intercellular communication among
8 various cell types was analyzed and reconstructed using the CellChat package (v
9 1.6.1)[46] to investigate the signaling interactions mediated by these key cell types.

10 **Reverse transcription quantitative PCR (RT-qPCR)**

11 A total of five pairs of tumor and adjacent normal tissue samples were collected at
12 Shenzhen People's Hospital, including five HCC and five adjacent non-tumor controls.
13 Prior to participation, all subjects were required to sign an informed consent form, and
14 the ethical approval was LL-KY-2022378, by the Medical Ethics Committee of
15 Shenzhen People's Hospital. TRIzol reagent was used to extract total RNA from 5
16 pairs of tissues (Ambion, USA). Subsequently, RNA quantification was conducted
17 utilizing the NanoPhotometer N50 instrument. Next, the SweScript First Strand cDNA
18 synthesis kit (Servicebio, China) was utilized to reverse transcribe mRNA to
19 synthesize cDNA. To perform RT-qPCR, the mixture included 3 μ L of cDNA
20 template, 5 μ L of SYBR Green Master Mix (2 $\times$), and 1 μ L of each primer (10 μ M
21 concentration). Primer sequences are listed in **Table 1**. Ultimately, the acquired data
22 were analyzed using the widely accepted $2^{-\Delta\Delta C_t}$ method, with GAPDH serving as the
23 internal reference gene to ensure accurate normalization.

1 **Table 1.** Primer sequences used for RT-qPCR

Primers		Sequence
FABP5	F	AGGAGTGGGAATAGCTTTGCG
FABP5	R	GCTGAACCAATGCACCATCT
FABP6	F	AGATCGTGGGTGACAAGCTG
FABP6	R	TTGGTGGGTTTGTAGCTCCC
MMP1	F	AGCCATCACTTACCTTGCACT
MMP1	R	CTGGGAAGCTGTGAGACACC
SGPP2	F	CAAGCCCGCTGAATCTCTCC
SGPP2	R	GAGGATCAACACAATTCCCACT
UGT1A10	F	GCCCCGTTTCCTTTATGTGTGT
UGT1A10	R	ATCTTCCAGAGTGTACGAGGTT
GAPDH	F	CGAAGGTGGAGTCAACGGATT
GAPDH	R	ATGGGTGGAATCATATTGGAAC

2

3 **Statistical analysis**

4 R software (v 4.1.0) was used to conduct statistical analyses. Group comparisons were
 5 conducted with Wilcoxon test, while Spearman correlation was applied to assess the
 6 association strength between variables. A $P < 0.05$ was regarded as statistically
 7 significant.

8

9 **Results**

1 Identification of candidate genes

2 In TCGA-LIHC cohort, 4,390 DEGs were found. Comparative analysis revealed that
3 1,198 genes were upregulated, while 3,192 genes were downregulated in HCC tumor
4 tissues versus adjacent normal tissues(**Figure 1a-b**). This investigation yielded 23
5 ESRGs that associated with HCC, among them, 16 genes, namely PLXNA2,
6 GALNT18, LAMA4 and so on, were high risk genes ($HR > 1$). While KDR, PLVAP,
7 TMEM204, TM4SF18, CYYR1, MMRN2, LMO2 were low risk genes ($HR < 1$)
8 (**Figure 2a**). In the TCGA-HCC cohort, a significant survival difference was observed
9 between groups with high and low scores ($P < 0.05$), with the high-score group
10 showing markedly better survival outcomes (**Figure 2b**). To explore the key module
11 genes, the investigation analyzed the genes' expression patterns through WGCNA. As
12 demonstrated in **Figure 2c-d**, WGCNA verification revealed the absence of any
13 substantial outlier samples. Subsequently, the optimal β was determined to be $\beta = 12$,
14 with $R^2 = 0.85$ (**Figure 2e**). Based on this, 13 co-expression modules were identified
15 with a scale-free network (**Figure 2f**). This study revealed three modules for which
16 the co-expression coefficient between ESRGs and the modules, including red module
17 (correlation coefficient (cor) = 0.84, $P < 0.05$), green module ($cor = 0.4$, $P < 0.05$) and
18 blue module ($cor = 0.33$, $P < 0.05$). Red module contained 786 genes, green module
19 contained 970 genes, and blue module contained 2,924 genes. In total, 4,680 key
20 module genes related to ESRGs were identified (**Figure 2g**). In this investigation, 52
21 candidate genes were identified by intersecting 4,390 DEGs, 4,680 key module genes
22 by WGCNA and 1,045 LMRGs (**Figure 2h**).

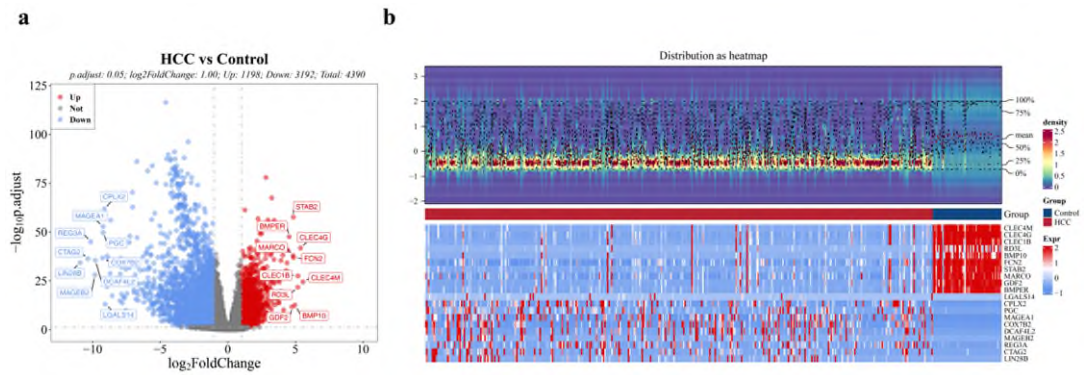


Figure 1. The DEGs analysis of TCGA-LIHC. (a) Volcano plot showing the distribution of DEGs between HCC and control groups. Red dots represent upregulated genes, blue dots represent downregulated genes, and gray dots represent genes with no difference. (b) Heatmap of DEGs between HCC and control. The image consists of two parts: the upper part shows the expression density heat map of the top 10 upregulated and downregulated DEGs, displaying five quantiles and the mean value; the lower part shows the expression heat map of the top 10 upregulated and downregulated DEGs.

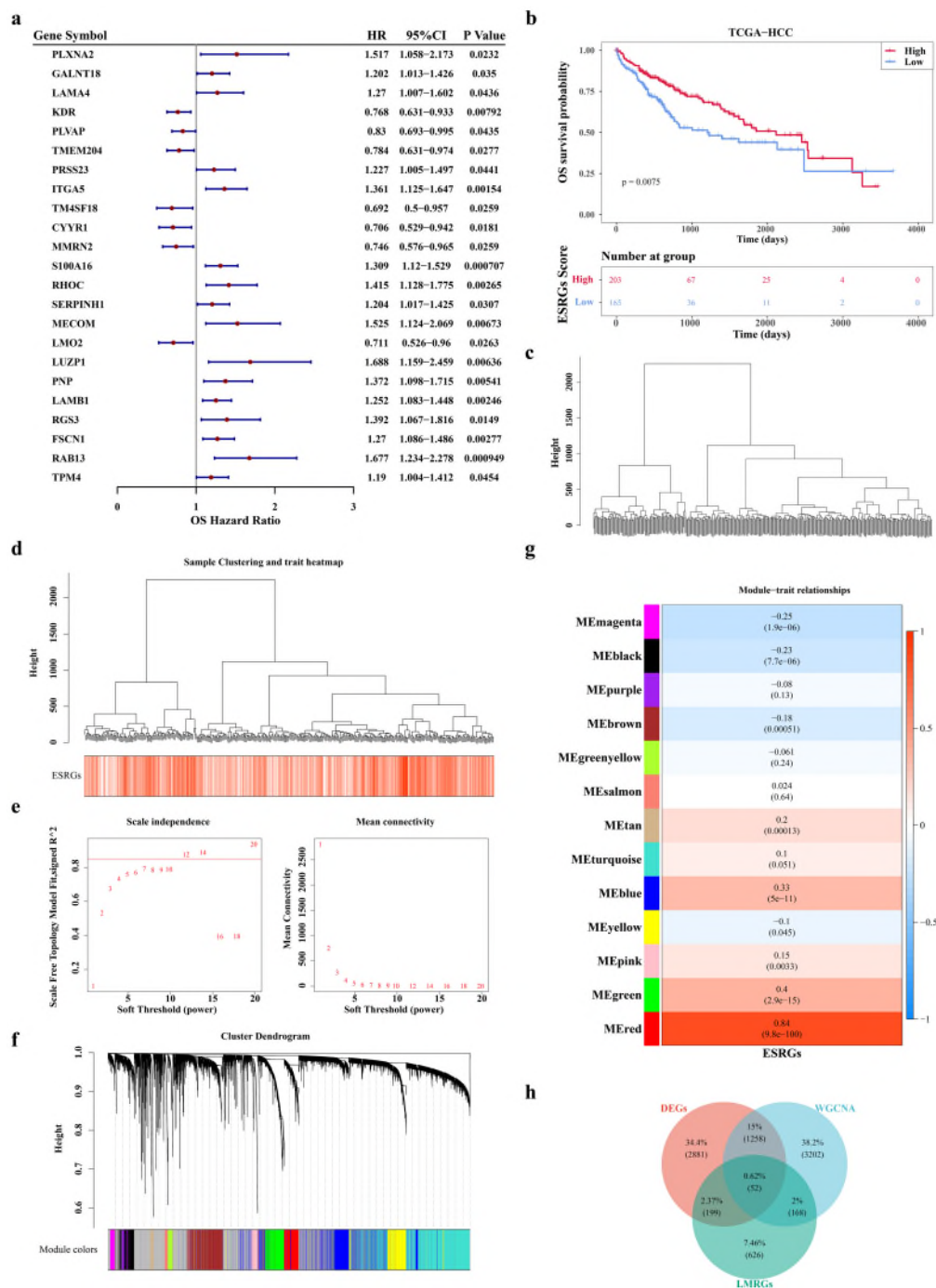


Figure 2. Identification of candidate genes. (a) ES-related prognostic gene forest plot, abbreviated as the risk ratio. (b) KM survival curves for high and low ESRG scores in the training set. (c) Sample-level clustering, where each branch in the clustering tree represents a sample, and the vertical axis represents the Euclidean distance between sample expression levels. (d) After introducing sample characteristics, sample-level

1 clustering. (e) Soft threshold screening: the optimal soft threshold is primarily
2 determined by referring to the left figure, which shows the scale-free fit index (y-axis)
3 under different soft threshold conditions (x-axis). The red line indicates the selected
4 value of the scale-free fit index. As shown in the left figure, the minimum soft threshold
5 corresponds to a scale-free fit index of 0.85. The right figure displays the network
6 connectivity for different soft threshold values. (f) Co-expression module identification.
7 This figure can be divided into two parts: the upper part is a hierarchical clustering tree
8 diagram of genes, and the lower part is a gene module, which is also a network module.
9 Corresponding to each other, we can see that genes that are close to each other
10 (clustered into the same branch) are divided into the same module. (g) Heatmap
11 showing the correlation between modules and phenotypes. The color blocks on the far
12 left represent modules, and the color bars on the far right represent the correlation range.
13 In the heat map in the middle, darker colors indicate higher correlations, with red
14 indicating positive correlations and blue indicating negative correlations. The numbers
15 in each cell indicate the correlation and significance. (h) Intersection gene.

16 **Functional analysis of candidate genes**

17 Functional enrichment analysis was conducted on 52 candidate genes to uncover their
18 biological roles. Specifically, 196 GO enrichments were obtained, including 169
19 biological processes (BP), like fatty acid metabolic process, lipid catabolic process,
20 lipid localization, and 27 molecular functions (MF) like calcium-dependent
21 phospholipid binding, acyltransferase activity, and phospholipase activity (**Figure 3a**).
22 A total of 24 KEGG pathways were enriched, like arachidonic acid metabolism
23 (**Figure 3b**). The PPI network of the candidate genes had 35 nodes and 37 edges

(Figure 3c). Among them, PTGS2 interacted most strongly with PLA2G4D, PLA2G4E, SPHK1 and PLA2G4F.

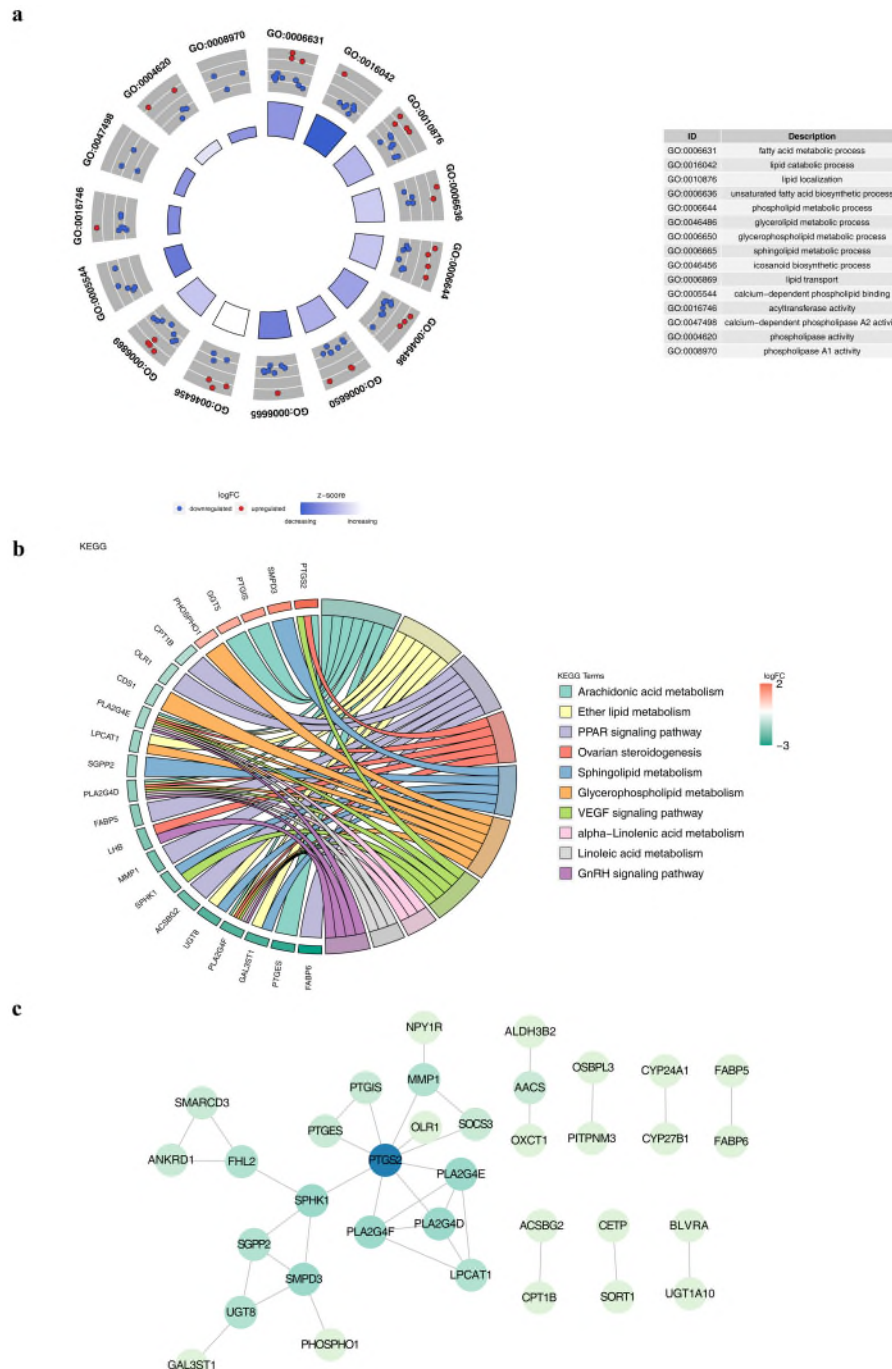


Figure 3. Functional analysis of candidate genes. (a) GO enrichment results circle diagram for candidate genes. (b) KEGG enrichment results and chord diagram for candidate genes. (c) PPI map of candidate genes, with different colors representing degree size.

1 **Construction and analysis of the prognostic gene signatures**

2 Five survival-associated genes were obtained by univariate Cox regression analysis
3 from 52 candidate genes, which were FABP5, FABP6, MMP1, SGPP2, and
4 UGT1A10 (HR > 1, P < 0.001) (**Figure 4a**). Subsequently, LASSO analysis identified
5 a panel of prognostic genes, including FABP5, FABP6, MMP1, SGPP2, and
6 UGT1A10, based on the minimum lambda value (0.0519) (**Figure 4b**). The specific
7 formula for the risk model was: Riskscore = MMP1 × (0.1854) + FABP6 × (0.0779) +
8 FABP5 × (0.0376) + SGPP2 × (0.0743) + UGT1A10 × (0.0112). Patients with HCC
9 were categorized into HRG and LRG, utilizing the optimal median risk score
10 threshold (median = 2.731). The number of deaths increased significantly with the
11 increase of the risk score in the samples (**Figure 4c-d**). KM survival curves depicted
12 significantly disparities in HRG and LRG, with the former exhibiting a diminished
13 survival probability (**Figure 4e**). Subsequent ROC analyses at 1, 2, and 3 years had an
14 AUC greater than 0.60, indicating moderate discriminative performance for these
15 prognostic genes (**Figure 4f**). Furthermore, the risk model was validated within
16 GSE15654 dataset, which demonstrated a worse prognosis for survival among HRG
17 (**Figure 4g-i**). Notably, AUC values exceeding 0.6 were observed for forecasting
18 survival over 1, 2, and 3 years(**Figure 4j**). It was revealed the risk model's
19 commendable predictive ability for OS in HCC.

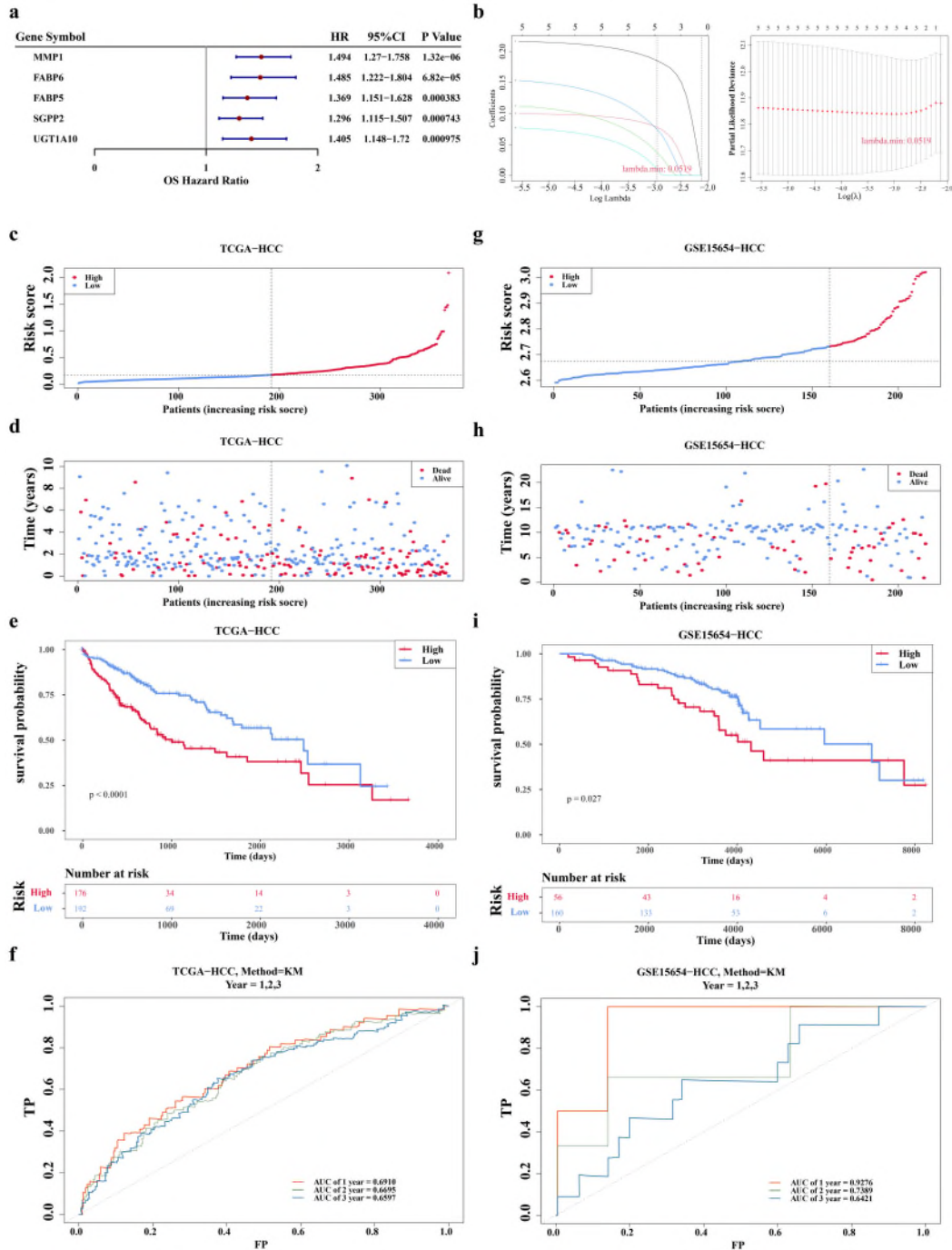


Figure 4. Construction and analysis of the prognostic gene signatures. (a) Single-factor Cox regression analysis forest plot, where HR represents the hazard ratio, abbreviated as the risk ratio. (b) Lasso model construction, left figure: coefficient spectrum, with the horizontal axis representing the logarithm of lambdas and the vertical axis representing variable coefficients. Right figure: Ten-fold cross-validation of adjustment parameters, with the horizontal axis representing the logarithm of lambdas and the vertical axis

1 representing model error. From the above figure, it can be seen that the optimal lambda
2 value is at the lowest point of the red curve, corresponding to the final number of genes.
3 (c) Risk curves for HRG and LRG of HCC patients in the training set. (d) Scatter plot of
4 HRG and LRG of HCC patients in the training set. (e) Survival curves for HRG and
5 LRG in the training set HCC, with red representing the HRG and blue representing the
6 LRG. (f) ROC curves for HCC patients in the training set at 1, 2, and 3 years. (g)
7 Survival curves for HRG and LRG of HCC patients in the validation set, with red
8 representing the HRG and blue representing the LRG. (h) ROC curves for HCC
9 patients in the validation set at 1, 2, and 3 years. (i) Risk curves for HRG and LRG of
10 HCC patients in the validation set. (j) Scatter plot of HRG and LRG of HCC patients in
11 the validation set.

12 **Analysis of independent prognostic factors and construction of nomogram**

13 Further investigation into clinicopathologic characteristics and risk scores in
14 prognostic models was conducted. The finding revealed that both risk score and T
15 stage with $P < 0.05$, met the PH assumption test ($HR > 1$) (**Figure 5a-b**).
16 Subsequently, nomogram predicting 1-, 2-, 3- year survival in HCC patients was
17 established (**Figure 5c**). The calibration curves demonstrated the prediction
18 probabilities were highly consistent with reference line (**Figure 5d**). Moreover, the
19 predictive performance for 1-, 2-, and 3-year survival was reflected by AUC values
20 greater than 0.6 (**Figure 5e**). In summary, these results validated the high predictive
21 accuracy for nomogram. A significant difference in risk scores was observed between
22 subgroups of T stage clinical characteristics within TCGA-HCC dataset. The T3-T4
23 stage exhibited a notably elevated risk score in comparison to the T1-T2 stages ($P <$
24 0.05) (**Figure 5f**).

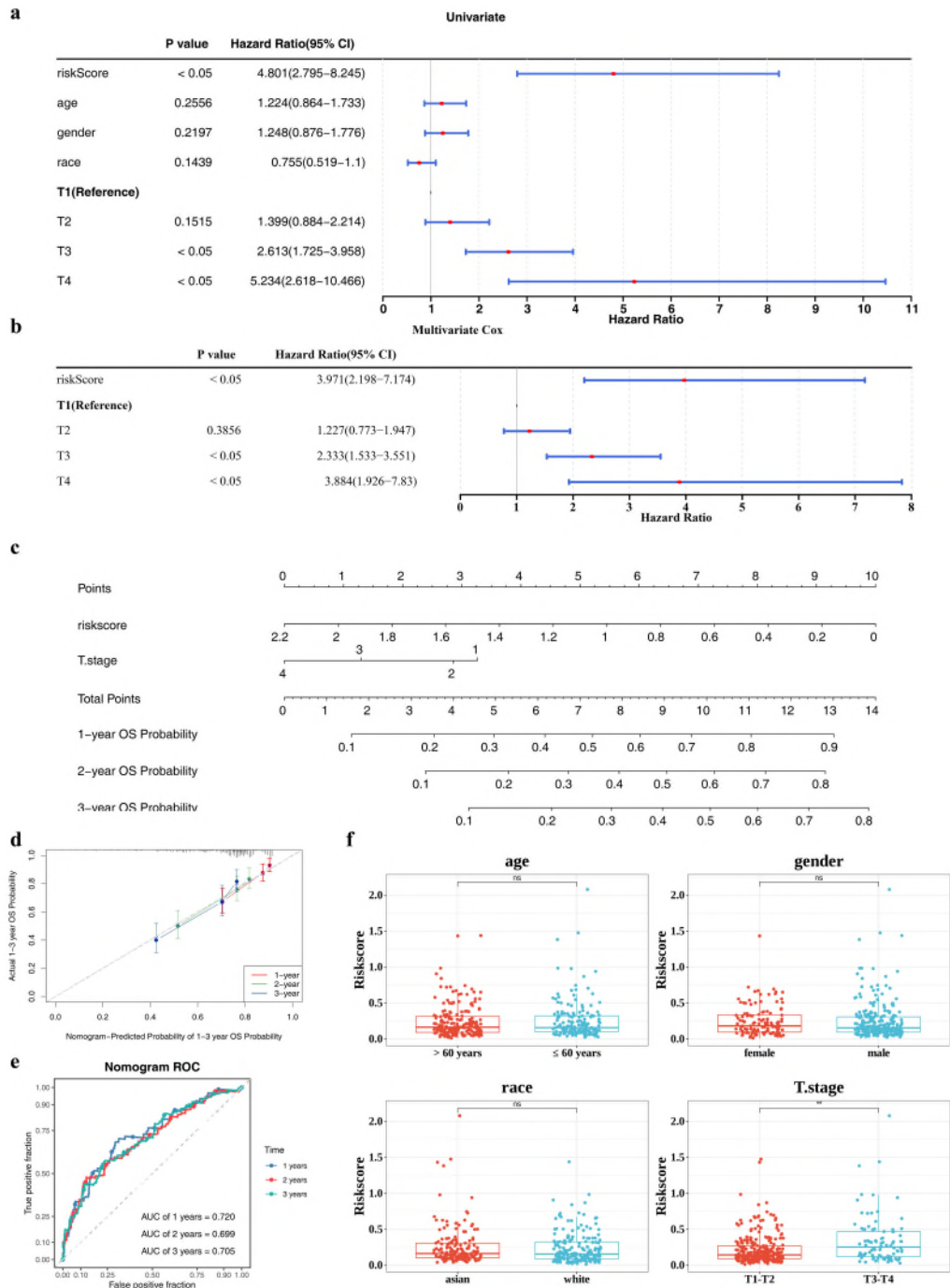


Figure 5. Analysis of independent prognostic factors and construction of nomogram.

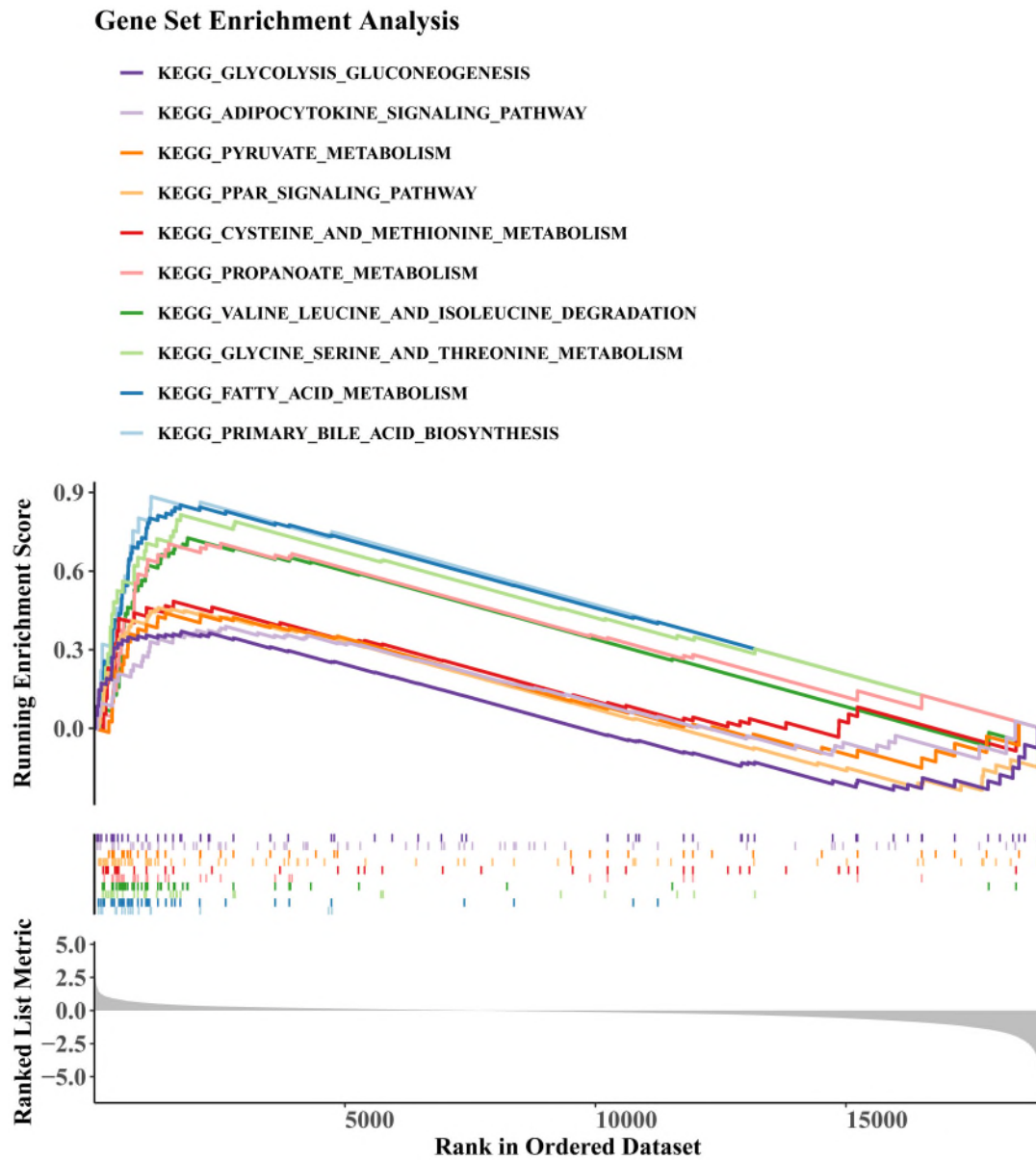
(a-b) HCC's univariate and multivariate Cox regression analysis of clinical characteristics and risk scores. The left figure shows the univariate independent prognostic analysis forest plot, and the right figure shows the multivariate independent prognostic analysis forest plot. (c) Constructing a survival nomogram for HCC patients based on prognostic models and clinical characteristics. (d) The calibration curve of the

1 clinical feature nomogram, with the horizontal axis representing the probability of
2 different clinical outcomes predicted by the model and the vertical axis representing the
3 probability of actual clinical outcomes observed in patients, is characterized by the
4 median plus the mean. (e) ROC curve of clinical characteristics. (f) Differences in risk
5 scores among clinical feature subtypes.

6 **Distinct metabolic pathways and immune landscape between HRG and LRG**

7 A comprehensive GSEA analysis revealed 34 pathways that were enriched in both the
8 HRG and LRG, including PPAR signaling pathway and cysteine and methionine
9 metabolism (**Figure 6**). This indicated a significant enhancement in metabolic
10 pathways, including lipid metabolism and glycolytic gluconeogenesis. The
11 distribution of 28 infiltrating immune cell types between HRG and LRG was assessed
12 and displayed in a heat map based on their enrichment scores (**Figure 7a**). Further
13 analysis revealed notable disparities in the levels of 18 immune cell types across the
14 two groups, such as eosinophils (**Figure 7b**). The Spearman correlation analysis
15 illuminated that all five prognostic genes exhibited a significant correlation with
16 various differentially immune infiltrating cells, with FABP5 demonstrating the
17 strongest positive correlation specifically with activated CD4 T cells ($\text{cor} = 0.52$, $P <$
18 0.05) (**Figure 7c**). Furthermore, a significant association was found between
19 differentially immune-infiltrating cells, with the macrophages and MDSC displaying
20 the strongest positive correlation ($\text{cor} = 0.85$, $P < 0.05$) (**Figure 7d**). The analysis
21 uncovered significant difference in 41 immune checkpoints between HRG and LRG,
22 such as TNFRSF18, TNFRSF4 and TNFRSF9 (**Figure 7e**). Furthermore, the results
23 demonstrated a significant elevation in the immune score ($P < 0.01$) and the Estimate
24 score ($P < 0.01$) within HRG compared to LRG (**Figure 7f**). Furthermore, when
25 evaluating the efficacy of immunotherapy responses, it was evident that the HRG

1 surpassed the LRG notably in TIDE, Dysfunction and Exclusion ($P < 0.01$) (**Figure**
 2 **7g**). These findings suggested a potential role for immune-related factors in
 3 determining response to immunotherapy.



4
 5 **Figure 6.** HRG and LRG KEGG enriched signaling pathways.

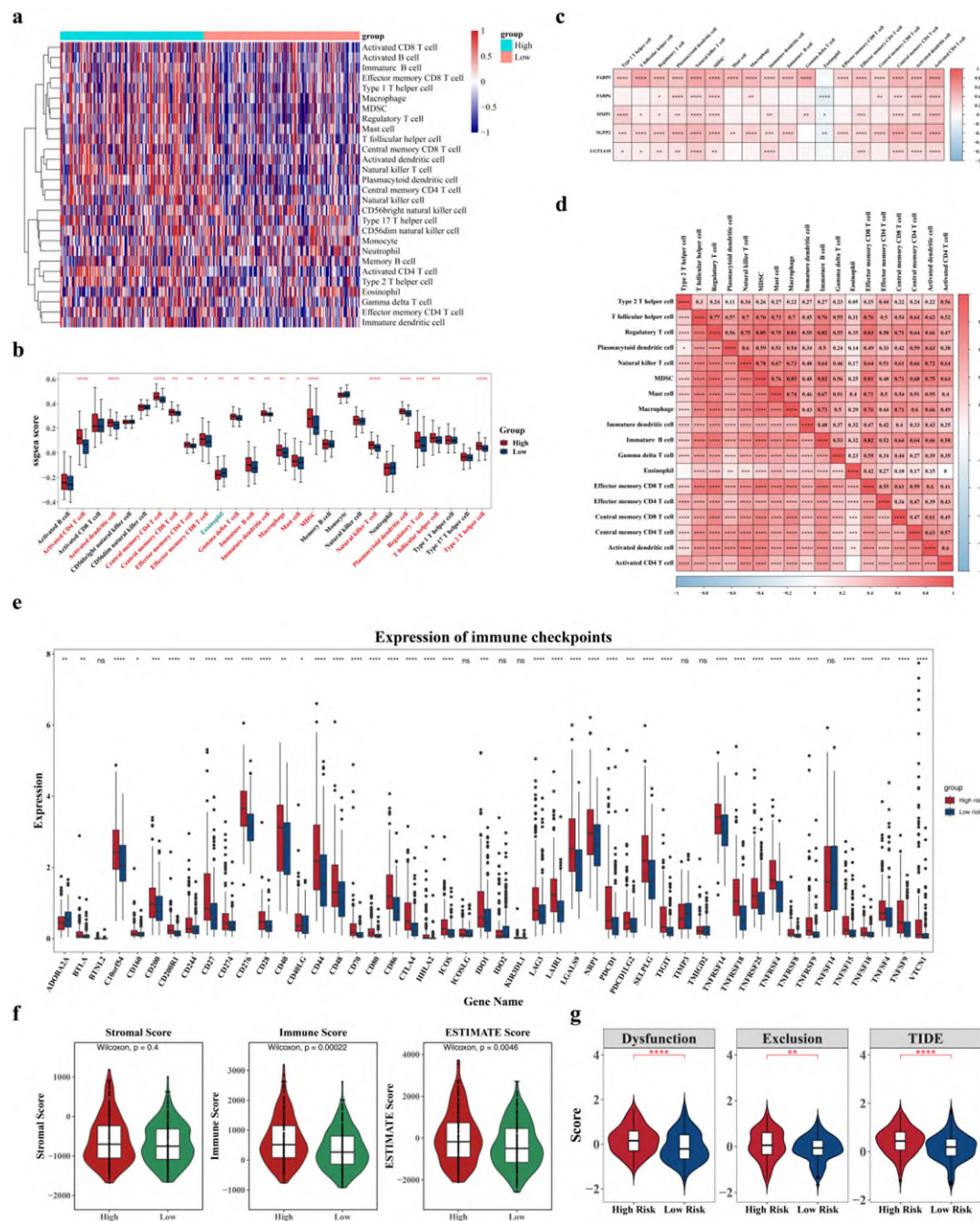


Figure 7. Immune cell distribution and correlations in HCC risk groups. (a) Immune infiltrating cell enrichment fraction based on heat map between HRG and LRG. (b) The abundance of 28 types of immune-infiltrating cells based on box plots between HRG and LRG. (c) Heatmap showing the correlation between prognostic genes and differential immune cells. (d) Heatmap showing correlations between different immune cells. (e) Differences in immune checkpoints between HRG and LRG. (f) The matrix score, immune score, and estimate score in the HRG and LRG of the training set. The

1 box plot shows the distribution of the matrix score, immune score, and estimate score in
2 the HRG and LRG. (g) Violin plot showing differences in TIDE scores between HRG
3 and LRG.

4 **Mutational profiles, TMB and drug response heterogeneity across HRG and** 5 **LRG**

6 The investigation revealed that both the HRG and LRG exhibited a preponderance of
7 missense mutations, with the TP53 having the highest mutation rate (43%) in the
8 HRG (**Figure 8a**), whereas the CTNNB1 gene had the most frequent mutation rate
9 (34%) in the LRG (**Figure 8b**). The assessment of the TMB scoring model revealed
10 that individuals in the low TMB group exhibited a markedly elevated probability of
11 survival (**Figure 8c**). Thereby the results demonstrated the better outcome of tumor
12 immunotherapy in low-TBM group. Besides, the mutation analysis of prognostic
13 genes highlighted a notable enhancement of mutations in FABP5, constituting 13% of
14 the total mutations (**Figure 8d**). The investigation uncovered a substantial difference
15 in the IC₅₀ values of 88 drugs between the HRG and LRG. Notably, patients in HRG
16 exhibited a more favorable response to all 58 common chemotherapeutic drugs, such
17 as A.443654, ABT.263 and ABT.888, evidenced by lower IC₅₀ values compared to
18 those in LRG. Conversely, their response to 30 other common chemotherapeutic
19 drugs was inferior, such as AS601245, Axitinib and AZD.0530, reflected in higher
20 IC₅₀ values than those observed in LRG (**Figure 9a-b**).

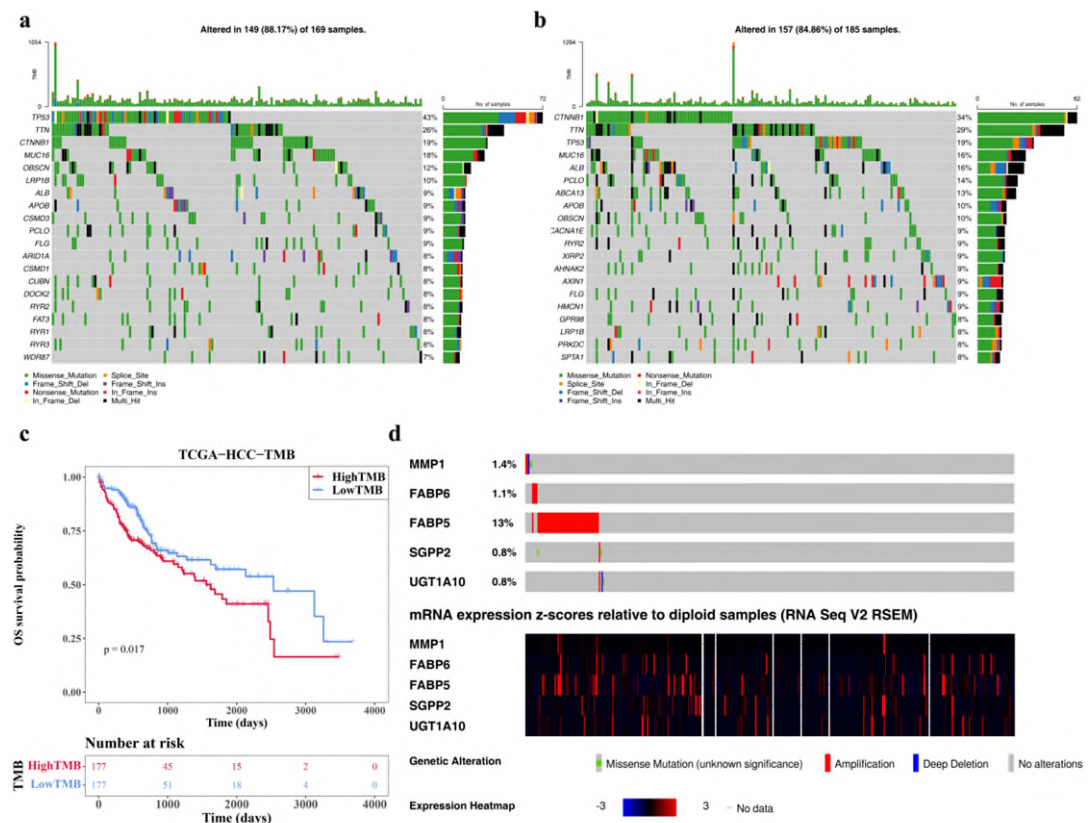


Figure 8. Analysis of somatic mutation. (a-b) HRG and LRG mutation waterfall plots, with a mutation heatmap in the middle. The color of each block in the heatmap corresponds to a mutation type, with the corresponding mutation types listed in the legend below. These include missense mutations, frameshift deletion mutations, nonsense mutations, frameshift insertion mutations, splice sites, intron insertions, intron deletions, transcription start sites, and stop codon mutations. (c) Survival differences between the high TMB group and the low TMB group. (d) Mutational information of prognostic genes generated using cBioPortal [47, 48] from the TCGA-LIHC cohort [49].

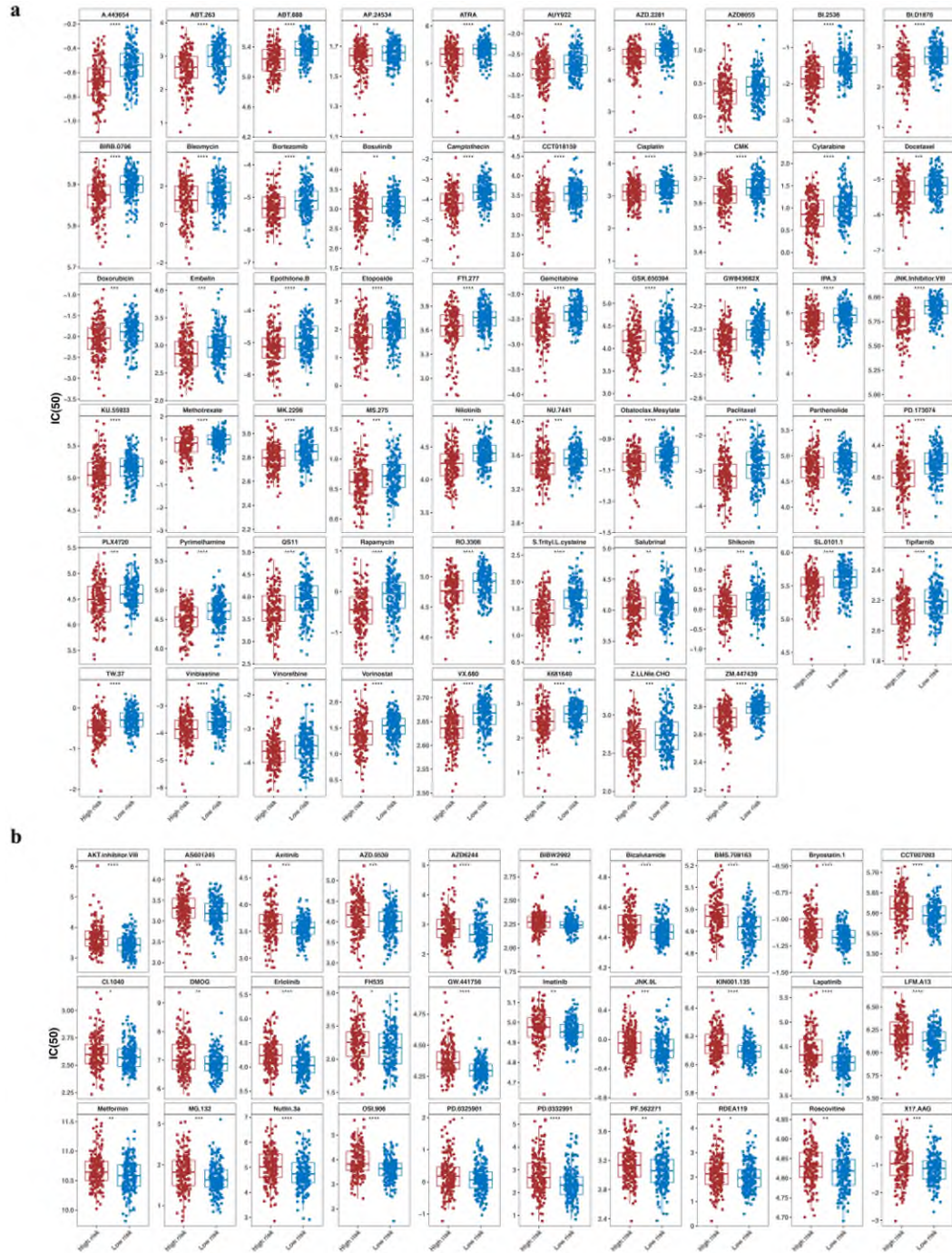
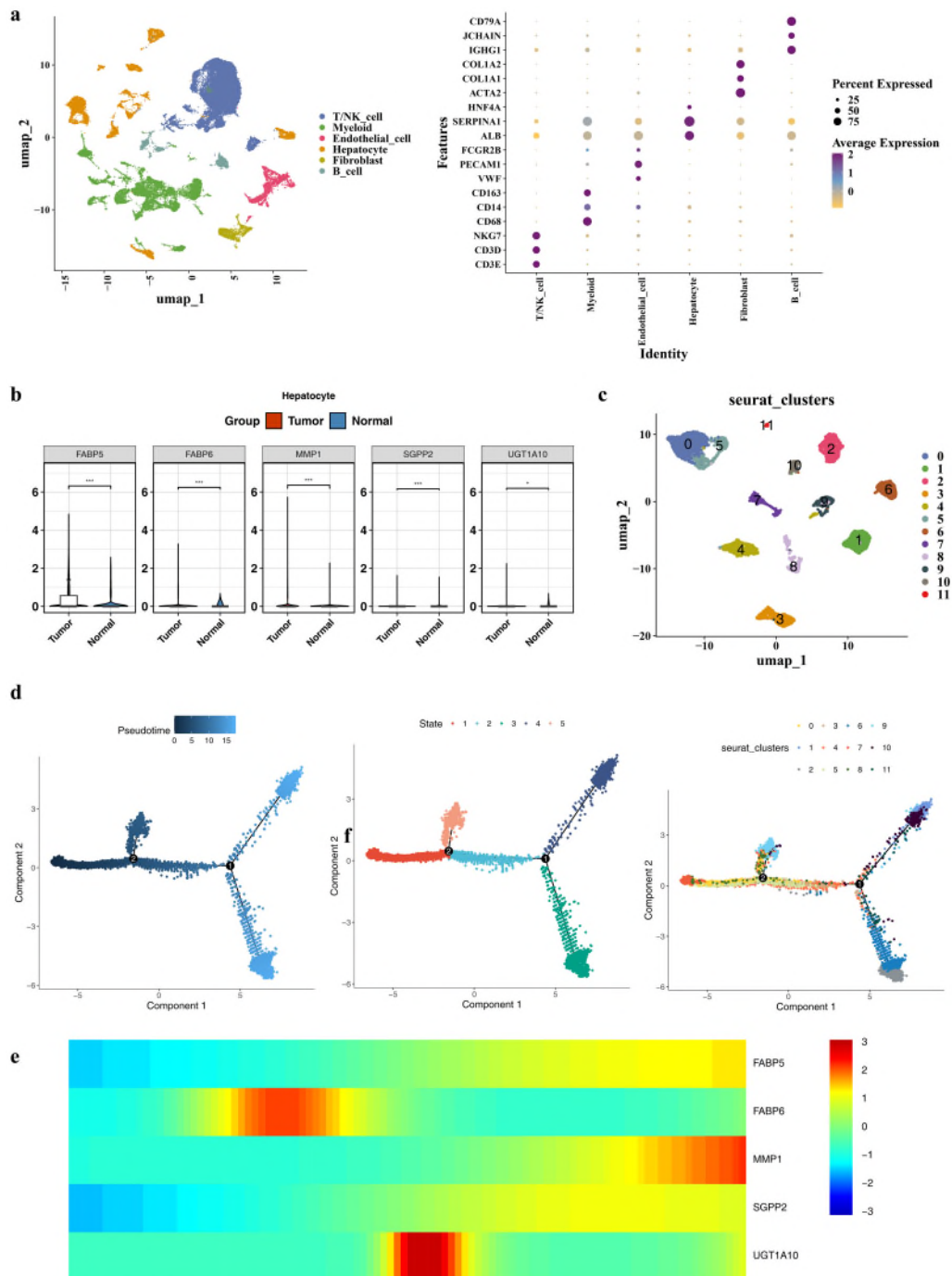


Figure 9. Analysis of drug sensitivity in HRG and LRG. (a) IC₅₀ (Down) of chemotherapy drugs between HRG and LRG. (b) Differences in chemotherapy drugs between HRG and LRG (Up).

Prognostic genes display hepatocyte-predominant and stage-specific expression dynamics in the HCC tumor microenvironment

1 To elucidate the cell-type-specific roles of the identified prognostic genes, scRNA-seq
2 analysis was performed on tumor and paired adjacent non-tumor tissues. Following
3 stringent quality control and dimensionality reduction (see **Additional file 1, Figure**
4 **S1a-d**), a total of 23 cell clusters were identified (see **Additional file 1, Figure S1c**).
5 These clusters were subsequently annotated into seven major cell lineages based on
6 canonical marker genes: T/NK cells, myeloid cells, monocytes, endothelial cells,
7 hepatocytes, fibroblasts, and B cells (**Figure 10a**). Single-cell differential expression
8 analysis revealed that all five prognostic genes—FABP5, FABP6, MMP1, SGPP2,
9 and UGT1A10—were expressed at significantly higher levels in hepatocytes derived
10 from tumor tissue compared with those from adjacent non-tumor tissue ($P < 0.05$)
11 (**Figure 10b**), suggesting that dysregulation of these genes might be predominantly
12 confined to the hepatocyte compartment. Accordingly, hepatocytes were prioritized
13 for more granular investigation. Re-clustering of the hepatocyte population yielded 12
14 distinct sub-clusters (**Figure 10c**). To delineate transcriptomic transitions within the
15 tumor microenvironment, pseudotime trajectory analysis was performed, defining five
16 discrete developmental states, in which state 1 was designated as the root, and states 3
17 and 4 were characterized as late-stage phenotypes (**Figure 10d**). Dynamic expression
18 profiling along the pseudotime axis revealed gene-specific patterns among the
19 prognostic genes. Specifically, FABP5, MMP1, and SGPP2 expression was
20 progressively upregulated as cells transitioned toward advanced developmental states,
21 whereas FABP6 and UGT1A10 displayed non-monotonic trajectories: FABP6 peaked
22 during the early-to-mid transition, and UGT1A10 reached maximal expression at the

1 mid-stage (**Figure 10e**). These findings indicated that the five prognostic genes
 2 exhibited stage-specific expression dynamics during hepatocyte malignant
 3 transformation, potentially reflecting their distinct functional contributions at different
 4 stages of HCC progression.



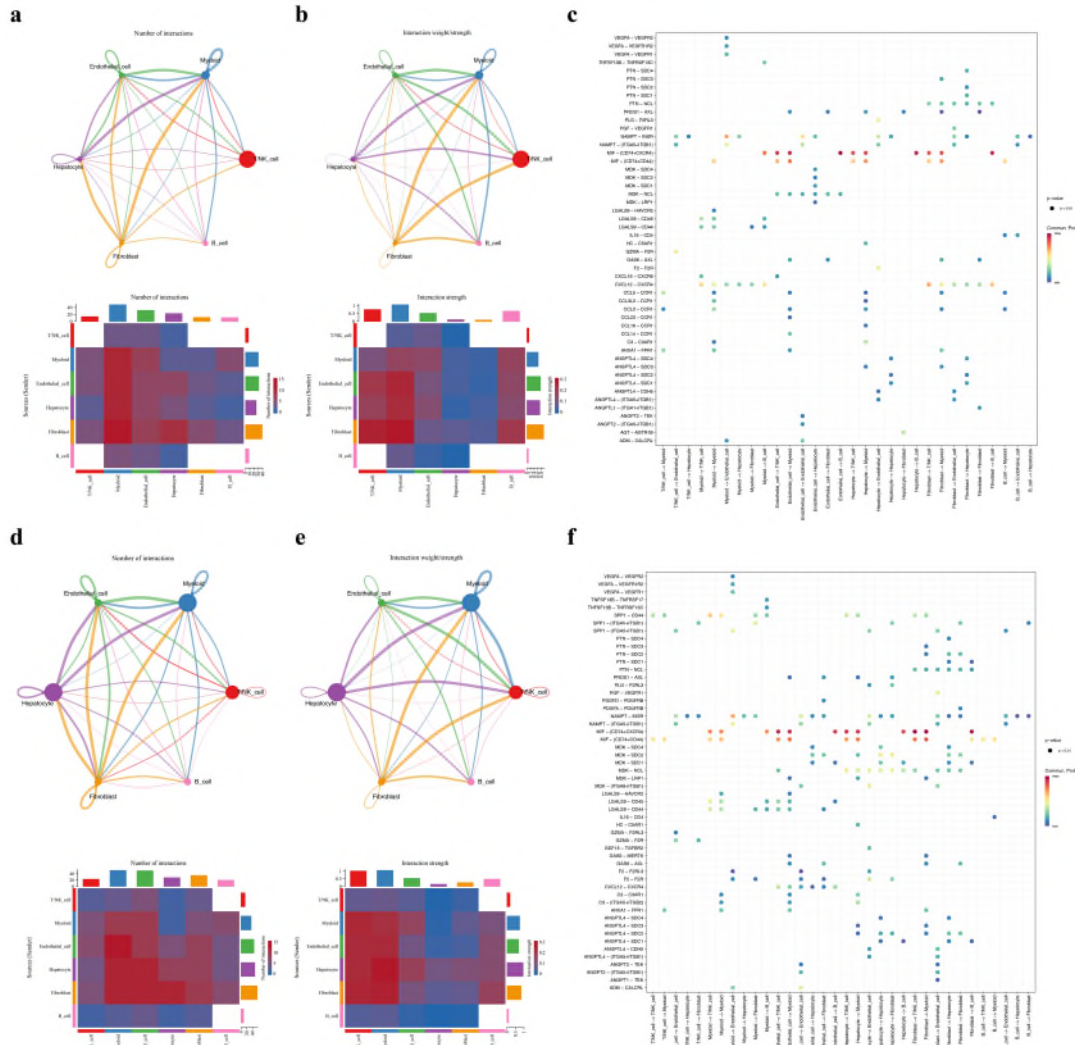
5
 6 **Figure 10.** Hepatocyte-predominant expression and pseudotime-resolved dynamics of

1 prognostic genes in the HCC tumor microenvironment. (a) UMAP plots showing the
2 annotation of seven major cell lineages. (b) The expression levels of the five prognostic
3 genes (FABP5, FABP6, MMP1, SGPP2, and UGT1A10) in hepatocytes derived from
4 tumor versus adjacent non-tumor tissues. (c) UMAP visualization of re-clustered
5 hepatocytes. (d) Pseudotime trajectory of hepatocyte sub-clusters constructed. (e)
6 Dynamic expression profiles of the five prognostic genes along the pseudotime axis.

7 **Remodeling of the HCC immune microenvironment**

8 To map the intercellular communication landscape within the HCC tumor
9 microenvironment, cell-cell communication analysis was performed on tumor and
10 paired adjacent non-tumor tissues. In non-tumor tissues, frequent crosstalk was
11 observed between hepatocytes and myeloid cells, while myeloid cells also interacted
12 extensively with endothelial cells and fibroblasts (**Figure 11a-b**). Within this network,
13 the NAMPT–(ITGA5+ITGB1) signaling axis was identified as a key mediator of
14 hepatocyte-to-immune signaling, facilitating communication from hepatocytes to B
15 cells and myeloid cells, from endothelial cells to T/NK cells, myeloid cells, and B
16 cells, and from fibroblasts to B cells (**Figure 11c**). In tumor tissues, the core
17 communication network encompassing hepatocytes, myeloid cells, fibroblasts, and
18 endothelial cells was maintained, albeit with markedly altered interaction intensities
19 (**Figure 11d-e**). Notably, the NAMPT–(ITGA5+ITGB1) signaling axis was
20 substantially expanded within the tumor microenvironment: in addition to the
21 interactions observed in non-tumor tissues (**Figure 11f**). Collectively, these findings
22 indicate that tumor-associated remodeling of the NAMPT signaling network is

1 accompanied by a broadening of its target cell repertoire, suggesting a potential role
 2 in shaping the immunosuppressive microenvironment that may favor HCC
 3 progression.



4
 5 **Figure 11.** Tumor-associated remodeling of intercellular communication networks in
 6 the HCC microenvironment. (a-b) Circle plot depicting the overall intercellular
 7 communication network in adjacent non-tumor tissues, heatmap summarizing the
 8 number and strength of inferred interactions between all cell type pairs in non-tumor
 9 tissues. (c) The cell-type-specific communication in non-tumor tissues. (d-e) Circle plot
 10 depicting the overall intercellular communication network in tumor tissues, heatmap

1 summarizing the number and strength of inferred interactions between all cell type
2 pairs in tumor tissues. (f) The expanded NAMPT–(ITGA5+ITGB1) signaling network
3 in tumor tissues.

4 **Regulatory network construction and RT-qPCR verification of candidate** 5 **prognostic genes**

6 A total of 120 miRNAs were obtained by taking the intersection of miRNet database
7 and NetworkAnalyst database. Additionally, 258 lncRNAs were obtained in this
8 investigation. Subsequently, a mRNA-miRNA-lncRNA regulatory network was
9 constructed, encompassing 5 mRNAs, 56 miRNAs (such as hsa-mir-31-5p,
10 hsa-mir-99a-5p and hsa-mir-145-5p), and 258 lncRNAs (such as MIR29B2CHG,
11 LINC01806 and SNHG4) (**Figure 12a**). The TF-mRNA network comprised 5
12 mRNAs and 24 TFs (like JUN, PPARG, MYC, and CTCF). Notably, UGT1A10 and
13 MMP1 both predicted PPARG, while MMP1 and FABP5 predicted MYC, and FABP6
14 and FABP5 both predicted CTCF (**Figure 12b**). The investigation revealed complex
15 regulatory connections within the network. Differences in the protein expression
16 levels of the five prognostic genes (FABP5, FABP6, MMP1, SGPP2, and UGT1A10)
17 were observed in HCC patients compared with normal subjects (**Figure 12c-g**).

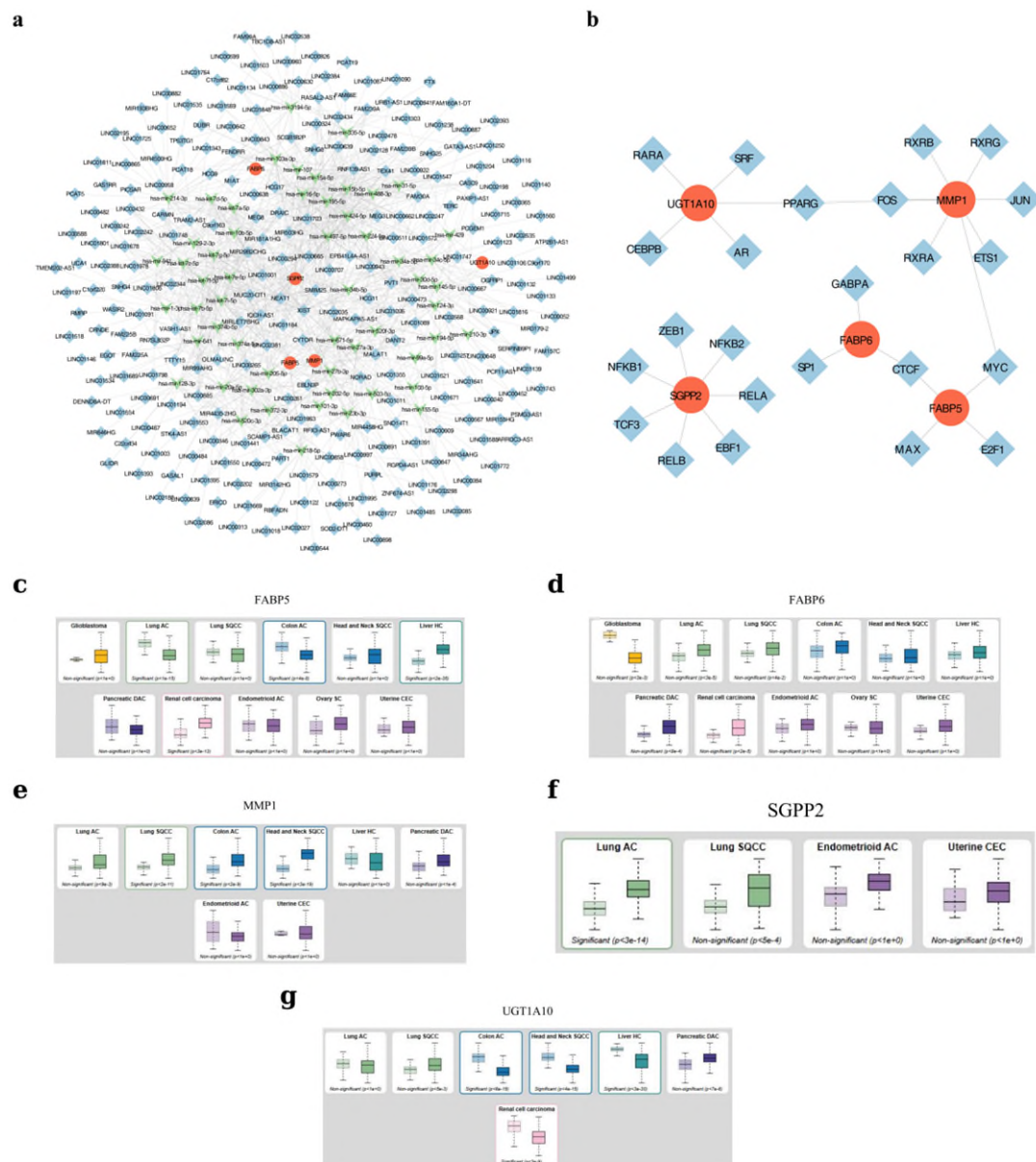


Figure 12. Construction of complex regulatory networks. (a) IncRNA-miRNA-mRNA regulatory network, red indicates prognostic genes, green indicates miRNAs, and blue indicates IncRNAs. (b) TF-mRNA regulatory network, red indicates key genes, and blue indicates transcription factors. (c–g) Protein expression patterns of FABP5, FABP6, MMP1, SGPP2, and UGT1A10 in HCC and normal liver tissues. Images reproduced from the Human Protein Atlas (image credit: Human Protein Atlas) under the CC BY 4.0 license [50–55].

Finally, the results obtained from RT-qPCR analysis were partly consistent with the

transcriptomic findings, highlighting a significant increase in the expression levels of FABP5, FABP6 and UGT1A10 in HCC samples ($P < 0.05$). In contrast, a significant decrease in the expression of MMP1 and SGPP2 was observed in HCC samples ($P < 0.05$) (**Figure 13**).

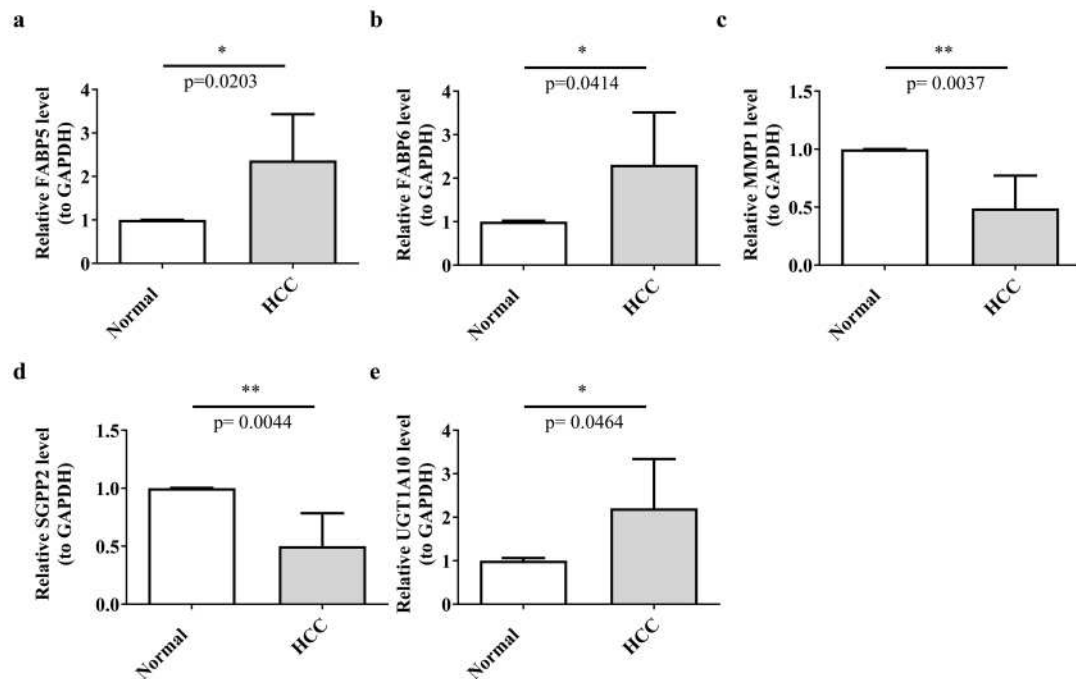


Figure 13. RT-qPCR verification. (a-e) Relative mRNA expression of FABP5, FABP6, MMP1, SGPP2, and UGT1A10, respectively, in five paired HCC and adjacent non-tumor tissues.

Discussion

Endothelial cell senescence and lipid metabolic reprogramming have both been implicated in HCC development [7, 8, 14], yet their shared prognostic relevance remains incompletely understood [10, 16, 23]. In the present study, five genes jointly associated with endothelial cell senescence and lipid metabolism—FABP5, FABP6, MMP1, SGPP2, and UGT1A10—were incorporated into a prognostic signature. The

signature stratified overall survival in the TCGA-LIHC cohort and retained prognostic discrimination in the external GSE15654 cohort. However, the 1-, 2-, and 3-year area under the curve values were only greater than 0.60, indicating modest rather than high predictive performance. RT-qPCR performed in five paired HCC and adjacent non-tumor tissues showed increased expression of FABP5, FABP6, and UGT1A10, but decreased expression of MMP1 and SGPP2. Among the five genes, FABP5 was prioritized for further interpretation because it showed the strongest positive correlation with activated CD4⁺ T-cell infiltration and displayed hepatocyte-predominant and stage-dependent expression patterns in the single-cell analysis.

FABP5 encodes an intracellular lipid-binding protein involved in lipid transport, inflammatory regulation, and cell proliferation [56]. Previous studies have identified FABP5 as an immunometabolic marker in HCC [57], linked it to obesity-associated hepatocarcinogenesis [24], and shown that FABP5-activated monocytes can increase programmed death ligand 1 expression on regulatory T cells [58]. Fatty acid-induced FABP5 expression has also been associated with hypoxia-inducible factor 1 activation and increased proliferation of liver cancer cells [59]. These observations are consistent with the increased FABP5 expression detected in the present study and support a potential role for FABP5 at the interface between lipid metabolism and immune regulation. Recent evidence suggests that FABP5 may couple tumor-cell lipid handling to myeloid-cell and T-cell remodeling, providing a plausible immunometabolic context for the FABP5-activated CD4⁺ T-cell association observed

1 here[22, 60]. The predicted association between FABP5 and CCCTC-binding factor in
2 the transcription factor network may provide an additional hypothesis, because
3 CCCTC-binding factor participates in chromatin organization and transcriptional
4 regulation and has been implicated in HCC growth, metastasis, and
5 methylation-associated transformation [61–63]. Nevertheless, this computationally
6 inferred relationship does not demonstrate direct regulation and requires experimental
7 validation.

8 The other four genes may provide complementary biological information within the
9 prognostic signature. FABP6, also known as ileal bile acid-binding protein, is
10 primarily involved in intracellular bile-acid handling and has been associated with
11 HCC progression, unfavorable survival, invasion, and angiogenesis [58, 63–70].
12 MMP1 is involved in extracellular matrix remodeling and has been linked to HCC
13 invasion and poor prognosis [71–73]. Although the regulatory network suggested a
14 possible relationship between MMP1 and FOS-family transcriptional regulation, this
15 finding should be regarded as hypothesis-generating because the present study did not
16 establish a direct MMP1–FOS regulatory mechanism [74]. SGPP2 participates in
17 sphingosine-1-phosphate dephosphorylation and inflammatory signaling, and its
18 expression can be induced under inflammatory conditions through nuclear factor
19 kappa B-related regulation [75–77]. UGT1A10 is a phase II drug-metabolizing
20 enzyme involved in glucuronidation, detoxification, and the metabolism of
21 endogenous compounds [78–85]. Collectively, these genes connect lipid handling,
22 extracellular matrix remodeling, inflammation, and xenobiotic metabolism, which

1 may partly explain their joint prognostic value in HCC.

2 The experimental findings also highlight the need for cautious interpretation of
3 gene-expression direction. FABP5, FABP6, and UGT1A10 showed tumor-associated
4 increases that were concordant between the single-cell and RT-qPCR analyses. By
5 contrast, MMP1 and SGPP2 were expressed at higher levels in tumor-derived
6 hepatocytes in the single-cell dataset but were downregulated in the five paired HCC
7 tissues examined by RT-qPCR. Several factors may contribute to this discrepancy.
8 First, the RT-qPCR cohort was small and may not capture the clinical and molecular
9 heterogeneity of HCC. Second, bulk tissue measurements reflect mixed cell
10 populations, whereas single-cell analysis evaluates expression within specific cellular
11 compartments. Differences in stromal, immune, endothelial, and hepatocyte
12 proportions may therefore influence the overall tissue-level signal. Third, variation in
13 disease stage, etiology, tumor differentiation, sampling location, and technical
14 platform may also affect expression estimates. Accordingly, the expression patterns
15 and prognostic implications of MMP1 and SGPP2 should not be considered
16 definitively established and require validation in larger, clinically characterized
17 cohorts.

18 Functional enrichment analysis further placed the five-gene signature within a
19 metabolic context. Gene set enrichment analysis indicated differences involving fatty
20 acid metabolism and peroxisome proliferator-activated receptor (PPAR) signaling
21 between the two risk groups. PPAR family members participate in hepatic lipid
22 homeostasis, energy balance, and inflammatory regulation [86–88]. Experimental

1 studies have also linked PPAR-related pathways to fatty acid synthesis, HCC
2 progression, the tumor immune microenvironment, and responses to anticancer agents
3 [87, 89–92]. These findings are consistent with the lipid-metabolism basis used to
4 derive the prognostic genes. Nevertheless, pathway enrichment reflects coordinated
5 transcriptional patterns rather than direct pathway activation, and the present results
6 do not establish that the five genes regulate PPAR signaling. Functional perturbation
7 studies will be needed to determine whether these genes participate directly in
8 PPAR-related metabolic reprogramming in HCC.

9 Among the immune-related findings, FABP5 showed the strongest positive
10 correlation with activated CD4⁺ T-cell infiltration. This association is biologically
11 plausible because FABP5 regulates intracellular lipid handling, whereas lipid
12 availability within the tumor microenvironment can influence T-cell activation and
13 function [93–95]. The reported ability of FABP5-activated monocytes to increase
14 programmed death ligand 1 expression on regulatory T cells [58] further raises the
15 possibility that FABP5-related lipid metabolism may participate in immune escape.
16 However, activated CD4⁺ T cells have heterogeneous and context-dependent functions.
17 They may support antitumor immunity by producing interleukin 2 and interferon
18 gamma and by enhancing the activity of CD8⁺ T cells, natural killer cells, and
19 macrophages [96, 97], whereas specific helper T-cell subsets may also contribute to
20 angiogenesis, immunosuppression, or the recruitment of myeloid-derived suppressor
21 cells [98–101]. Activated CD4⁺ T-cell infiltration was higher in the high-risk group,
22 consistent with previous observations [102], but the present correlation cannot

1 determine whether FABP5 promotes immune suppression, reflects compensatory
2 immune activation, or is indirectly associated with both processes. FABP5-specific
3 perturbation combined with immune-cell functional assays is therefore required.

4 The single-cell analysis provided further information on the cellular origin and
5 temporal expression of the prognostic genes. All five genes were predominantly
6 expressed in hepatocytes and were more highly expressed in tumor-derived
7 hepatocytes than in adjacent non-tumor hepatocytes. Pseudotime analysis showed
8 distinct expression trajectories: FABP5, MMP1, and SGPP2 increased toward later
9 cellular states, whereas FABP6 and UGT1A10 peaked at earlier or intermediate states
10 [5, 103–107]. These patterns suggest that the genes may be associated with different
11 hepatocyte state transitions during HCC evolution. However, pseudotime represents
12 an inferred ordering of cellular states rather than a direct longitudinal observation, and
13 it cannot establish that these genes drive malignant transformation. A liver-disease
14 single-cell study reported enhanced NAMPT–ITGA5/ITGB1 signaling associated
15 with vascular remodeling, providing contextual support for our CellChat finding
16 without establishing an HCC-specific causal link[108]. CellChat analysis additionally
17 suggested expansion of the NAMPT–(ITGA5+ITGB1) signaling axis in tumor tissues,
18 involving a broader range of recipient cell types, including T/NK cells [46,109–111].

19 This finding supports altered hepatocyte-centered intercellular communication in the
20 HCC microenvironment, but a direct mechanistic connection between FABP5, the
21 five-gene signature, and this signaling axis remains to be demonstrated.

22 Several limitations should be considered. First, the study was retrospective and relied

1 primarily on public transcriptomic datasets. Although the prognostic signature was
2 evaluated in an external cohort, the modest area under the curve values indicate that
3 its incremental clinical value remains uncertain. Second, the RT-qPCR analysis
4 included only five paired samples, limiting the robustness of the experimental
5 validation and the interpretation of discordant MMP1 and SGPP2 expression. Third,
6 potential confounders related to HCC etiology, treatment, stage, and molecular
7 subtype were not comprehensively evaluated. Fourth, the immune-cell correlations,
8 regulatory networks, pseudotime trajectories, and CellChat interactions were
9 computational inferences without direct functional confirmation. Finally, the model
10 should be compared with established clinical variables and prognostic systems in
11 larger multicenter cohorts before its clinical applicability can be considered.

12

13 **Conclusions**

14 In conclusion, this study developed and externally evaluated a five-gene prognostic
15 signature linking endothelial cell senescence and lipid metabolism in HCC. Integrated
16 bulk transcriptomic, immune, single-cell, intercellular communication, and RT-qPCR
17 analyses further prioritized FABP5 as a candidate associated with lipid metabolic
18 alterations, hepatocyte state transitions, and activated CD4⁺ T-cell infiltration. These
19 findings provide hypothesis-generating evidence rather than proof of a causal
20 mechanism or immediate clinical utility. Future studies should assess the stability and
21 added prognostic value of the signature and determine whether FABP5 directly
22 influences lipid metabolism, CD4⁺ T-cell responses, or hepatocyte-centered

1 intercellular communication in HCC.

2

3

4 **List of abbreviations**

Abbreviation	Full Term
HCC	hepatocellular carcinoma
EL	endothelial cell senescence and lipid metabolism
ELRGs	EL-related genes
ESRGs	endothelial cell senescence-related genes
LMRGs	lipid metabolism-related genes
RT-qPCR	reverse transcription quantitative polymerase chain reaction
HBV	hepatitis B virus
HCV	hepatitis C virus
TCGA	the Cancer Genome Atlas
GEO	Gene Expression Omnibus
DEGs	differentially expressed genes
KM	Kaplan-Meier
IC ₅₀	half-maximal inhibitory concentration
WGCNA	weighted gene co-expression network analysis
HRG	high-risk group
LRG	low-risk group

Abbreviation	Full Term
AUC	area under the curve
ROC	receiver operating characteristic
FDR	false discovery rate
NES	normalized enrichment score
LASSO	least absolute shrinkage and selection operator
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
FABP5	fatty acid binding protein 5
PD-L1	programmed death ligand 1
FABP6	fatty acid-binding protein 6
SGPP2	sphingosine-1-phosphate phosphatase 2
UGT1A10	UDP-glucuronosyltransferase 1A10
MMP1	matrix metalloproteinase 1
scRNA-seq	single-cell RNA sequencing
PPI	protein-protein interaction
TMB	tumor mutation burden
TIDE	tumor immune dysfunction and exclusion
HPA	Human Protein Atlas
TF	transcription factor
miRNA	microRNA
lncRNA	long noncoding RNA

Abbreviation	Full Term
NAMPT	nicotinamide phosphoribosyltransferase
ITGA5	integrin subunit alpha 5
ITGB1	integrin subunit beta 1
PPAR	peroxisome proliferator-activated receptor
UMI	unique molecular identifier
HVG	highly variable gene
PCA	principal component analysis
UMAP	uniform manifold approximation and projection

1 **Declarations**

2 **Ethics approval and consent to participate**

3 The study was conducted in accordance with the Declaration of Helsinki and was
4 approved by the Medical Ethics Committee of Shenzhen People's Hospital (No.
5 LL-KY-2022378; June 19, 2023). Written informed consent was obtained from all
6 participants before sample collection.

7 **Consent for publication**

8 Not applicable.

9 **Availability of data and materials**

10 The publicly available datasets analyzed in this study are available from the Genomic
11 Data Commons (TCGA-LIHC: <https://portal.gdc.cancer.gov/projects/TCGA-LIHC>)
12 and the Gene Expression Omnibus (GSE15654:
13 <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE15654>; GSE149614:

1 <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE149614>). The RT-qPCR
2 data generated in this study are available from the corresponding author on reasonable
3 request.

4 **Competing interests**

5 The authors declare that they have no competing interests.

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8 commercial, or not-for-profit sectors.

9 **Authors' contributions**

10 MX: Conceptualization, Data curation, Validation, Visualization, Writing–original
11 draft, Writing–review & editing. ZL: Data curation, Validation, Visualization,
12 Writing–review & editing. HZ: Validation, Writing–review & editing. LHL:
13 Visualization, Writing–review & editing. LPL: Conceptualization, Project
14 administration, Supervision, Writing–review & editing. This work currently described
15 has not been published, is not being considered for publication elsewhere, and its
16 publication was approved by all authors.

17 **Acknowledgements**

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19 who supported and assisted us throughout this research. Without your support, this
20 research would not have been possible.

21

22 **Additional files**

1 Additional file 1

2 File format: .tif

3 Title of data: Figure S1. Quality control and dimensionality reduction of scRNA-seq
4 data

5 Description of data: (a) Violin plots depicting the distribution of key quality-control
6 metrics across all cells. (b) The top 2,000 highly variable genes. (c) UMAP
7 visualization of 23 cell clusters and the canonical marker-gene expression used for
8 cell-type annotation. (d) Principal-component assessment used to select components
9 for downstream dimensionality reduction and clustering.

10 **Figure S1.** Quality control and dimensionality reduction of scRNA-seq data. (a) Violin
11 plots depicting the distribution of key quality control metrics across all cells. (b) The
12 top 2,000 HVGs were identified. (c) UMAP visualization of all cells after quality
13 control, displaying 23 distinct clusters. The accompanying canonical marker-gene dot
14 plot was used for cell-type annotation. (d) The optimal number of principal components
15 for downstream dimensionality reduction and clustering.

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