

Construction of a Prognostic Model for Pancreatic Cancer Based on mitochondrial dynamics-Related Genes: Application of Multiple Machine Learning Methods

Ma Gongping

Affiliated Hospital of Southwest Medical University

Liu Yuchuan

Affiliated Hospital of Southwest Medical University

Qin Shu

15883062343@163.com

Affiliated Hospital of Southwest Medical University

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Abstract

Purpose

To investigate the prognostic significance of mitochondrial dynamics-related genes in pancreatic adenocarcinoma (PAAD) and determine whether a machine learning-derived signature could predict patient survival, tumor microenvironment characteristics, and therapeutic response.

Methods

Transcriptomic and clinical data from TCGA-PAAD and normal pancreatic tissues from GTEx were analyzed to identify differentially expressed mitochondrial dynamics-related genes. Multiple machine learning algorithms were applied to construct a prognostic model, which was externally validated using ICGC and GEO cohorts. Survival, Cox regression, functional enrichment, somatic mutation, immune infiltration, and drug sensitivity analyses were performed. Single-cell RNA sequencing and spatial transcriptomics were used to characterize the cellular and spatial distributions of the core genes.

Results

Thirty-two prognostic mitochondrial dynamics-related genes were identified, of which seven genes—ALDH3A1, CD36, CKB, FOXM1, IGF2BP3, INS, and MET—were selected for the final signature. The resulting risk score effectively stratified patients into groups with significantly different survival outcomes and remained an independent prognostic factor after adjustment for clinicopathological variables. High-risk tumors were associated with cancer progression, cell-cycle and motility pathways, immunosuppressive features, distinct somatic mutations, and reduced sensitivity to several chemotherapeutic agents. Single-cell analysis localized multiple signature genes predominantly to ductal epithelial cells, while spatial analysis showed increasing MET expression during malignant progression.

Conclusion

The seven-gene mitochondrial dynamics signature provides a potential tool for prognostic stratification and therapeutic-response prediction in PAAD. Prospective clinical validation and functional experiments are required before clinical application.

Introduction

Pancreatic cancer, one of the most lethal malignancies of the digestive system, is renowned for its extremely high invasiveness and dismal prognosis. It ranks among the leading causes of cancer-related deaths worldwide. Due to the lack of early symptoms and effective early diagnostic markers, most pancreatic cancer patients are already at an advanced stage when diagnosed, with a 5-year survival rate of only about 9% (Siegel et al. 2024). Pancreatic ductal adenocarcinoma (PDAC) is the most common subtype of pancreatic cancer, accounting for over 90% of pancreatic malignancies, and its prognosis is particularly

poor. Although surgical resection remains the preferred treatment for PDAC patients, only 15–20% of patients have surgical indications at the time of diagnosis (Versteijne et al. 2022; Shaib et al. 2016). In recent years, new strategies such as FOLFIRINOX combination chemotherapy and neoadjuvant therapy have significantly improved survival rates for some patients (Conroy et al. 2011). However, the efficacy of chemotherapy regimens is limited by drug resistance (Beatty et al. 2021), highlighting the urgency of exploring novel adjuvant therapeutic strategies and biomarkers to improve prognostic assessment and guide personalized treatment.

Mitochondria, known as the "powerhouses" of the cell, are responsible for cellular respiration, oxidation of NADH and FADH₂, and biosynthesis of ATP, serving as the core of cellular energy metabolism (Nunnari and Suomalainen 2012). One of the key characteristics of mitochondria is their dynamic nature, i.e., mitochondrial dynamics, which encompasses a series of dynamic processes, including transport along the cytoskeleton, interaction with other subcellular structures, and the ability to alter the morphology of their inner membrane (IMM) and outer membrane (OMM) through fusion and fission events (Quintana-Cabrera and Scorrano 2023). Given the multiple physiological roles of mitochondrial dynamics, elucidating how changes in these processes affect critical aspects of tumor cell growth remains challenging. Numerous studies have shown that regulating mitochondrial fusion and fission activities can influence tumor metabolism, cell cycle, proliferation, migration, and even is closely related to the maintenance of cancer stem cells (Kashatus 2018; Maycotte et al. 2017). In pancreatic cancer, mitochondrial dynamics is involved in the complex molecular regulatory network of tumor cells. For instance, heat shock protein 70 (HSP70) mediates the phosphorylation of dynamin-related protein 1 (DRP1) via PTEN-induced kinase 1 (PINK1), thereby coordinating mitochondrial dynamics and autophagy and serving as a multifunctional driver of tumorigenesis (Ferretti et al. 2024). LncRNA UCA1 regulates mitochondrial dynamics in pancreatic cancer cells through the MAPK/ERK pathway to promote migration (Wang et al. 2023). Moreover, highly invasive and metastatic pancreatic cancer cells and pancreatic cancer stem cells (CSCs) are highly dependent on mitochondrial metabolism, and the mitochondrial fission process is a critical link for pancreatic CSCs to maintain their stemness (Courtois et al. 2021). Additionally, recent studies have found that mitochondrial fission can activate the cGAS-STING pathway through mtDNA release, remodeling the tumor immune microenvironment (West et al. 2015), while loss of the fusion protein OPA1 can enhance the sensitivity of PDAC to gemcitabine (Anderson et al. 2018), indicating that abnormal mitochondrial dynamics not only drive malignant phenotypes but are also directly related to immune evasion and drug resistance.

However, alterations in a single molecule are often insufficient to fully reflect the complexity of the mitochondrial dynamics network. Therefore, this study aims to utilize multiple machine learning methods to elucidate the important role of mitochondrial dynamics in the prognosis of pancreatic cancer and to investigate the somatic mutation characteristics in pancreatic cancer. We plan to construct a model for evaluating the prognosis of clinical pancreatic cancer patients and to explore the potential molecular mechanisms of mitochondrial dynamics in pancreatic cancer treatment and its value as a potential therapeutic target.

Materials and methods

2.1 Data Sources

This study selected clinical and pathological information (age, sex, tumor grade, tumor stage, tumor type, tumor location, TNM stage, survival days, survival status, etc.) and transcriptome sequencing data (seq-tpm, seq-count) from 178 pancreatic cancer samples from the public Cancer Genome Atlas (TCGA) database, and transcriptome sequencing data (seq-tpm, seq-count) from 180 normal pancreatic tissues from GTEx. All transcriptome sequencing data were normalized. Among them, one sample in TCGA without survival data was excluded, and 177 samples were finally included. mitochondrial dynamics-related genes were obtained from the GeneCards database (<https://www.genecards.org/>). ICGC-PACA-AU, GSE78229, GSE57495, and GSE62452 were obtained from the ICGC and GEO databases as external validation cohorts. Single-cell RNA sequencing data (scRNA-seq) GSE155698 (including 10 PAAD tumor tissues) were used for single-cell analysis. Immunohistochemistry (IHC) data were downloaded from the Human Protein Atlas (HPA, <http://www.proteinatlas.org>) to verify protein expression in PAAD and normal tissues. Spatial transcriptomics (ST) data GSE274557 were downloaded from the GEO database for analyzing the spatial and temporal distribution of genes.<https://www.genecards.org>/<http://www.proteinatlas.org>

2.2 Methods

2.2.1 Differential Gene Expression Analysis and Identification of mitochondrial dynamics-Related Differentially Expressed Genes

Based on the transcriptome data of TCGA-PAAD ($n = 177$) and GTEx normal pancreatic tissues ($n = 180$), we used the DESeq2 package in R to identify differentially expressed genes (DEGs) between tumor and non-tumor samples according to the criteria of $|\text{fold-change}| > 1.5$ and adjusted P value < 0.05 . The results were visualized using the "ggplot2" package.

From the GeneCards database (accessed on September 22, 2024), we collected a total of 644 mitochondrial dynamics-related genes. Using the VennDiagram package in R, an intersection analysis of PAAD DEGs and mitochondrial dynamics-related genes was performed, and 32 mitochondrial dynamics-related genes differentially expressed in PAAD were screened for subsequent analysis.

2.2.2 Construction and Validation of a Prognostic Model for mitochondrial dynamics-Related Genes Based on Multiple Machine Learning Methods

To construct a prognostic model based on the identified 32 mitochondrial dynamics-related genes (CARGs), we first performed univariate Cox regression analysis on these genes. Subsequently, multiple machine learning algorithms were applied, including StepCox (stepwise regression), Random Survival Forest (RSF), SuperPC (supervised principal component analysis), Lasso (least absolute shrinkage and selection operator), Survival-SVM (survival support vector machine), GBM (gradient boosting machine), CoxBoost (boosting algorithm for Cox regression), Enet (elastic net regression), Ridge (ridge regression), and plsRcox

(partial least squares regression for Cox model). The model construction was conducted using the "Mime1" package in R. Then, we established models based on single and combined machine learning algorithms and calculated the C-index for each model. Using the TCGA-PAAD, ICGC-PACA-AU, GSE78229, GSE57495, and GSE62452 datasets, we evaluated the predictive performance of each model and calculated their average C-index across these five datasets for visualization. Ultimately, the combination of Lasso and plsRcox was determined as the optimal model.

Based on the median risk score calculated by the optimal model combination as the cutoff, we divided the TCGA-PAAD training set into high-risk and low-risk groups. Using the "survival" and "survminer" packages, Kaplan-Meier survival curves were plotted, and the log-rank test was performed to assess the survival differences between the two groups. The "timeROC" package was used to plot receiver operating characteristic (ROC) curves and calculate the area under the curve (AUC) to evaluate the model's predictive accuracy in predicting 2-year, 3-year, and 5-year survival rates. In addition, GEO datasets were used to validate the stability and effectiveness of the CARGs prognostic model.

Subsequently, we applied univariate and multivariate Cox proportional hazards models to assess whether the CARGs prognostic model could independently predict patient prognosis after adjusting for other clinicopathological characteristics (such as age, sex, histological grade, pathological stage, and TNM stage). Based on univariate analysis, we integrated relevant risk factors to construct a nomogram for prognostic prediction. Calibration curves were plotted to evaluate the consistency between the model-predicted survival probabilities and the actual observed values.

2.2.3 Functional Enrichment Analysis of Differential Genes

Using the DESeq2 package in R, we identified differentially expressed genes (DEGs) between the high-risk and low-risk groups with screening criteria of $|\text{fold-change}| > 1$ and adjusted P value < 0.05 . Functional enrichment analysis of these DEGs was conducted using the "clusterProfiler" package to explore their potential biological functions. Gene set enrichment analysis (GSEA) was conducted based on "c2.cp.reactome.v7.0.entrez.gmt" and "h.all.v7.0.symbols.gmt" reference gene sets. Pathways were considered statistically significant when the nominal P value corresponding to the normalized enrichment score (NES) was < 0.05 and the false discovery rate (FDR) q -value was < 0.25 . The most enriched pathways were ranked based on the normalized enrichment score (NES). The limma package (version 3.54.2) was used to quantify the difference in pathway activity between the high-risk and low-risk groups.

2.2.4 Tumor Microenvironment and Immune Infiltration Analysis

We used the ssGSEA algorithm to calculate the abundance scores of 28 immune cell types from the gene expression data of the TCGA dataset, including activated B cells, activated CD4 + T cells, M1 macrophages, plasma cells, etc., to evaluate the tumor immune microenvironment. Based on the differential analysis results, we compared the infiltration levels of these immune cells between the high-risk and low-risk groups. Wilcoxon rank-sum test and Spearman correlation analysis were used to explore the relationship between

risk scores and immune checkpoint gene expression. The ESTIMATE algorithm was applied to calculate immune scores and stromal scores and to assess their correlation with the mitochondrial dynamics risk score. Results were visualized with box plots. The TIDE algorithm was used to evaluate the response of each sample to immunotherapy. With immunotherapy data from the TCIA database, we compared the immune scores of high- and low-risk groups under different immune checkpoint inhibitory genes (such as CD44 and TNFSF9). Results were displayed in box plots, and the Wilcoxon rank-sum test was used to assess the significance of differences.

2.2.5 Gene Mutation Analysis

Using the *maftools* package, we analyzed and visualized the somatic mutation data of the high-risk and low-risk groups in the TCGA dataset, comparing the mutation counts and mutation spectra between the two groups. Subsequently, the "*maftools*", "*remodeling2*", and "*ggpubr*" packages were used to generate waterfall plots showing the mutation distribution of key mutated genes in each group. Combining the mitochondrial dynamics risk score and tumor mutation burden (TMB) data, we compared the TMB differences between the high- and low-risk groups. A dot-line plot was drawn with the "*ggpubr*" package to display TMB differences, and statistical tests were performed. Based on the median TMB value, samples were further divided into high TMB and low TMB groups. The chi-square test was used to evaluate the proportional difference between high- and low-risk groups, and the results were shown in violin plots.

2.2.6 Drug Sensitivity Assessment

We used the *oncoPredict* package in R to evaluate the predictive ability of the mitochondrial dynamics risk score for chemotherapeutic and targeted drugs. In the TCGA-PAAD dataset ($n = 177$), we calculated the half-maximal inhibitory concentration (IC50) for multiple drugs for each patient and divided patients into high-risk ($n = 88$) and low-risk ($n = 89$) groups based on the risk score. Wilcoxon rank-sum test was used to analyze the difference in IC50 Z-scores between the high- and low-risk groups, exploring the potential application value of the mitochondrial dynamics risk score in predicting drug sensitivity. Results were displayed through box plots and scatter plots.

2.2.7 Single-cell RNA Sequencing Analysis and HPA Immunohistochemical Validation

Single-cell RNA sequencing (scRNA-seq) data were downloaded from the GEO database, including raw sequencing data of 10 PAAD tumor tissues (GSE155698). The *Seurat* package (v4.3.0.1) was used for data processing and analysis. Cells with gene counts (*nFeature_RNA*) between 200 and 5,000 were selected; cells with UMI counts (*nCount_RNA*) exceeding 50,000 or below 200 were removed; cells with *nFeature_RNA* between 200 and 5,000 were retained. Cells with mitochondrial UMI count (*pMT*) exceeding 20% and red blood cell UMI count (*pHB*) exceeding 5% were removed. Data normalization was conducted using the "*NormalizeData*" and "*ScaleData*" functions, and the "*FindVariableFeatures*" function was used to identify 2,000 highly variable genes. Dimensionality reduction was conducted through principal component analysis (PCA), and batch effects were corrected using the "*RunHarmony*" function. Cell populations were

visualized using t-SNE and UMAP methods, and cell clusters were manually annotated based on known marker genes.

2.2.8 Spatial Transcriptomics Analysis

Spatial transcriptomics (ST) data (GSE274557) were downloaded from the GEO database (GSE274557, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE274557>). This dataset was processed through an integrated computational pipeline: initial spot-level filtering based on *nCount_Spatial* and *nFeature_Spatial* thresholds to exclude low-quality regions, followed by SCTransform normalization (Seurat v5) and PCA-based feature selection (dims = 1:15). Unsupervised clustering (FindClusters, resolution = 0.8) and UMAP visualization were then used to reveal spatial domains. The RCTD algorithm, combined with GSE155698 scRNA-seq data, was implemented for cell type deconvolution and core gene localization. Furthermore, DDRTree pseudotime analysis was conducted using Monocle3 to reconstruct the ductal epithelial trajectory, and SpaCET combined with TCGA PAAD reference data was used to determine malignant cell distribution. Topological feature extraction was employed to identify tumor-stroma interfaces, and multi-core parallelization (8 threads) was applied to accelerate subclone refinement. Finally, statistical validation of the spatial co-localization network was conducted using permutation test (FDR < 0.05), and the final results were visualized with Cytoscape.<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE274557>

Statistical Analysis

All statistical analyses were performed using R software (version R 4.3.2) and corresponding R packages. All statistical tests were two-sided, and a P value less than 0.05 was considered statistically significant.

Results

3.1 Identification of Differentially Expressed Genes and mitochondrial dynamics-Related Genes in Pancreatic Cancer and Their Enrichment Analysis

(Fig-1 Differential gene analysis and enrichment analysis of PAAD samples using TCGA combined with GTEx. (a) DEGs in PAAD tumors (b) 32 differentially expressed key mitochondria dynamics-related genes (c) Principal component analysis (PCA) of the 32 differential genes (d) Biological process annotation of the 32 DEGs (e) Cellular component annotation of the 32 DEGs (f) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment of the 32 DEGs (g) Molecular function annotation of the 32 DEGs (h) Gene Ontology (GO) functional annotation of the 32 DEGs)

Based on the integrated TCGA and GTEx cohorts, we compared PAAD tumor samples with normal pancreatic tissues and performed differential expression analysis. This analysis identified a total of 4,625 differentially expressed genes (DEGs) meeting the criteria of P value < 0.05 and $|\text{fold-change}| > 1.5$ (Fig. 1a). Among them, 3,701 genes were up-regulated and 924 genes were down-regulated in tumor tissues.

To screen for mitochondrial dynamics-related genes, we obtained a list of 644 such genes from the GeneCards database (accessed on September 22, 2024). By intersecting this list with the identified DEGs, we selected 32 key mitochondrial dynamics-related genes that were differentially expressed in PAAD samples (Fig. 1b). To assess whether the expression profiles of these 32 genes could distinguish tumor from non-tumor samples, we performed principal component analysis (PCA). The PCA results showed a significant clustering separation between the two groups, indicating that these genes could effectively differentiate PAAD tissues from normal pancreatic tissues (Fig. 1c).

To explore the potential molecular mechanisms by which these 32 mitochondrial dynamics-related genes may contribute to PAAD progression, we performed Gene Ontology (GO) enrichment analysis. The results showed that these genes were mainly enriched in biological processes related to fatty acid metabolic processes and reactive oxygen species metabolism, suggesting that these metabolic processes may play an important role in pancreatic cancer-associated mitochondrial dynamics. In terms of cellular components, enrichment analysis indicated that these genes were closely associated with structures such as membrane rafts, membrane microparticles, and actin-based cell projection clusters (Fig. 1e). Furthermore, the enrichment was closely related to molecular functions such as creatine kinase activity and phosphotransferase activity. Overall, these findings suggest that mitochondrial dynamics may influence the development and progression of PAAD by controlling metabolism and motility-related cellular structures.

3.2 Construction and Validation of a Prognostic Model Based on mitochondrial dynamics-Related Prognostic Genes

We employed multiple machine learning methods to construct a risk prediction model based on mitochondrial dynamics-related genes in the TCGA database and validated it in the ICGC and GEO datasets. The model construction utilized both individual and combined machine learning algorithms, and the C-index was calculated for each model. Subsequently, the average C-index across the external validation datasets was calculated to evaluate the predictive performance of all models. Ultimately, we determined that the combination of Lasso (least absolute shrinkage and selection operator) and plsRcox (partial least squares regression for Cox model) (first using Lasso with 10-fold cross-validation to select features, then using partial least squares Cox regression) was the optimal model, with a C-index of 0.626 (Fig. 2a). Lasso was used for feature selection, screening important genes with a *P* value less than 0.05 (`unicox_p_cutoff = 0.05`) to reduce gene feature dimensionality, and then partial least squares regression (PLS) combined with Cox survival regression model was used to construct the survival prediction model. PLS analysis selected one principal component, and model parameters included node size (`nodesize = 5`) and random seed (`seed = 12345`). Finally, seven genes with significant impact on survival outcomes were identified: *ALDH3A1*, *CD36*, *CKB*, *FOXM1*, *IGF2BP3*, *INS*, *MET* (Fig. 2b).

(Fig-2 Machine learning-based risk prediction model set, scatter plots, survival curves, and ROC curves. (a) Risk prediction model set; (b) Seven genes with significant impact on survival outcomes; (c), Scatter

plot; (d,e) TCGA-PAAD; (f,j) GSE57495; (g,k) GSE62452; (h,l) GSE78229; (i,m) ICGC)

Based on this algorithm, we calculated the risk score for each sample ($\text{RiskScore} = -1.75455035 + 0.14827705\text{ALDH3A1} - 0.19396747\text{CD36} - 0.21103729\text{CKB} + 0.25205893\text{FOX M1} + 0.22937553\text{IGF2BP3} + 0.04094880\text{INS} + 0.40405226*\text{MET}$), and using the median risk score of 0.103158 as the threshold, pancreatic cancer (PAAD) patients were divided into low-risk group (better prognosis) and high-risk group (poor prognosis). Ultimately, 177 TCGA-PAAD patients were divided into a high-risk group ($n = 88$) and a low-risk group ($n = 89$). The scatter plot showed that as the individual risk score increased, overall survival (OS) significantly decreased (Fig. 2c,d). Kaplan-Meier survival curves and log-rank test further confirmed that the OS of patients in the high-risk group was significantly lower than that of the low-risk group ($p < 0.0001$; Fig. 2c).

To evaluate the performance of the prognostic model, we performed time-dependent ROC curve analysis to assess the sensitivity and specificity of the model. The AUCs for predicting 2-year, 3-year, and 5-year survival were 0.976, 0.987, and 0.938, respectively, indicating that the model has high accuracy in predicting OS (Fig. 2e). Next, we validated the feasibility of predicting the prognosis of PAAD patients based on the mitochondrial dynamics signature using three GSE datasets and one ICGC dataset. Kaplan-Meier survival curves showed that patients in the low-risk group had better OS than those in the high-risk group (Fig. 2e). In the risk score models of both training and validation sets, robust AUC values were observed at 2, 3, and 5 years (Fig. 2j-m). In summary, this mitochondrial dynamics-related prognostic signature based on multiple machine learning methods showed good performance in predicting the survival outcomes of PAAD.

3.3 Construction and Validation of the mitochondrial dynamics-Related Prognostic Signature as an Independent Prognostic Factor for PAAD

(Fig-3 Validation of independent prognostic factors of the mitochondrial dynamics-related risk score. (a) Stepwise Cox regression and nomogram. (b) AUC values for predicting 2-, 3-, and 5-year overall survival. Calibration curves for (c) 2-year, (d) 3-year, and (e) 5-year survival)

To evaluate whether the mitochondrial dynamics-related risk score is an independent prognostic factor for PAAD patients, we included it together with various clinical characteristics (age, sex, histological grade, T stage, M stage, N stage, history of malignancy, follow-up tumor status, tumor location, DCC tissue type, radiotherapy history, residual tumor status, and history of chronic pancreatitis) into a multivariate Cox regression analysis. Stepwise Cox regression was used to construct a nomogram to predict 2-year, 3-year, and 5-year overall survival (Fig. 3a). The parameters included in the nomogram were risk score, age, N stage, histological grade, follow-up tumor status, radiotherapy history, DCC tissue type, and residual tumor status. The results highlighted the importance of the mitochondrial dynamics-related risk score in the prognostic prediction of PAAD patients. The AUC values of the nomogram for predicting 2-year, 3-year, and 5-year overall survival reached 0.845, 0.857, and 0.901, respectively (Fig. 3b). Next, the precision of the nomogram model was evaluated by calibration curves. The calibration curves were highly consistent with the 45-degree diagonal line, indicating that the survival probabilities predicted by the model were in good agreement with the actual survival data in the TCGA-PAAD cohort (Fig. 3c-e).

3.4 Correlation Analysis of Immune Infiltration and Tumor Microenvironment

To further explore the relationship among mitochondrial dynamics, immune cell infiltration, and tumor microenvironment, ssGSEA (single-sample gene set enrichment analysis) was used to compare the immune cell infiltration between the high-risk and low-risk groups. The results showed that the infiltration levels of immune/inflammatory cells such as activated B cells, eosinophils, myeloid-derived suppressor cells, macrophages, mast cells, and monocytes were significantly higher in the low-risk group than in the high-risk group ($P < 0.001$), and the infiltration levels of immature B cells and CD8 + T cells were also higher in the low-risk group ($P < 0.01$). Additionally, the infiltration of immature dendritic cells, plasmacytoid dendritic cells, regulatory T cells, follicular helper T cells, and type I T helper cells was also more pronounced in the low-risk group ($P < 0.05$) (Fig. 4a). In terms of immune function, cytolytic activity, T cell co-stimulation, and type II interferon response were all significantly enhanced in the low-risk group ($P < 0.001$) (Fig. 4b). Further using the ESTIMATE.

(Fig-4 Immune infiltration and tumor microenvironment. (a) Immune cell infiltration (ssGSEA). (b) Immune function. (c) Immune checkpoint. (d) Tumor purity. (e) ESTIMATE score. (f) Immune score. (g) Stromal score. (h) TIDE score.)

algorithm, we analyzed the relationship between mitochondrial dynamics-related genes and the tumor microenvironment in the 177 patients of the TCGA-PAAD cohort. The analysis results showed that the tumor purity of the high-risk group was significantly higher than that of the low-risk group, while the stromal score, immune score, and ESTIMATE score were relatively lower in the high-risk group (Fig. 4d-g), suggesting reduced immune components in the tumor microenvironment of the high-risk group. In immune checkpoint gene expression analysis, BTLA, CD276, CD44, HHLA2, LGALS9, CD160, TNFSF15, and TNFSF9 were significantly upregulated in the high-risk group ($p < 0.01$) (Fig. 4c), suggesting that these immune-related molecules may be involved in the immunosuppressive state of the high-risk group, and the development of inhibitors targeting them may have clinical potential. In addition, to evaluate the potential response of patients in different risk groups to immune checkpoint blockade (ICB) therapy, we used the TIDE (Tumor Immune Dysfunction and Exclusion) tool. The TIDE score is closely related to tumor immune evasion and immunotherapy resistance; a higher TIDE score indicates a lower response rate to immunotherapy. The results showed that the potential responsiveness of pancreatic cancer patients to ICB therapy did not differ significantly between the risk groups, indicating that there may be no significant difference in response to conventional ICB treatment between the high- and low-risk groups (Fig. 4h).

3.5 Chemotherapy Response Prediction and CARGs Risk Score

(Fig-5 Prediction of chemotherapy drug response. Correlation between risk score and efficacy; prediction of drug response (IC50) in high- and low-risk groups. (a,b) Gemcitabine. (c,d) Cisplatin. (e,f) Oxaliplatin.

(g,h) Irinotecan. (l,j) Selumetinib. (k,l) Trametinib. (m,n) Ulixertinib. (o,p) Osimertinib. (q,r) Lapatinib. (s,t) Gefitinib. (u,v) Dasatinib. (w,x) Bortezomib. (y,z) Acetalax.)

We utilized cell line data from the Cancer Genome Project (CGP) database to predict the half-maximal inhibitory concentration (IC50) of commonly used chemotherapeutic drugs in PAAD patients from different risk groups. Using the oncoPredict package, the differences in IC50 of existing drugs between the high- and low-risk groups were calculated. The results showed that the sensitivity of the low-risk group to gemcitabine, a first-line chemotherapeutic drug for pancreatic cancer, was significantly higher than that of the high-risk group ($P < 0.001$), and the risk score was significantly positively correlated with the IC50 of gemcitabine (correlation coefficient = 0.27) (Fig. 5a,b). This means that the IC50 values in the low-risk group were significantly lower, suggesting greater sensitivity to gemcitabine, while patients in the high-risk group may be more prone to drug resistance. In addition, for other first-line chemotherapeutic drugs, such as platinum drugs (oxaliplatin, cisplatin) and topoisomerase inhibitors (irinotecan), the IC50 values in the low-risk group were also significantly lower than those in the high-risk group ($P < 0.001$, Fig. 5c-h). Notably, the IC50 values of the high-risk group for Selumetinib, Trametinib, Ulixertinib, Osimertinib, Lapatinib, Gefitinib, Dasatinib, Bortezomib, and Acetalax were significantly lower than those in the low-risk group (Fig. 5i-z), suggesting that these drugs may have potential therapeutic value for high-risk patients.

3.6 Functional Analysis of mitochondrial dynamics-Related Gene Sets

To explore the differences in biological behavior between different mitochondrial dynamics risk score groups, we performed functional annotation analysis on TCGA-PAAD samples using the GSVA R package. The heatmap of GSVA-KEGG analysis showed that pathways related to the cell cycle, homologous recombination, and cell junctions were significantly enriched in the high-risk group. In addition, cancer-associated pathways were also markedly enhanced in the high-risk group. Meanwhile, the P53 pathway showed higher activity in the high-risk group (Fig. 6a). Conversely, the low-risk group showed significant enrichment in amino acid metabolic pathways.

(Fig-6 Functional annotation analysis of mitochondrial dynamics-related gene sets. (a) GSVA-KEGG heatmap. (b) GSVA-HALLMARK enrichment analysis. (c) GO. (d) KEGG.)

GSVA-HALLMARK enrichment analysis further indicated that multiple pathways associated with cancer progression and cell proliferation were significantly enriched in the high-risk group. These pathways include MYC targets, DNA repair, G2/M checkpoint, PI3K/AKT/mTOR signaling pathway, hypoxia response, and apoptosis, among others (Fig. 6b). Notably, the activities of cancer-related pathways such as p53, Notch, TGF- β signaling pathways, and the PI3K/AKT/mTOR signaling pathway were also more prominent in the high-risk group. GSEA enrichment analysis revealed that the high-risk group was enriched in MET-related pathways such as cell motility, protein ubiquitination, programmed cell death, and cell cycle checkpoints.

To further explore the genetic differences between mitochondrial dynamics-related genes (MDAGs) and the risk scores of PAAD patients, we first identified differentially expressed genes (DEGs) between the high-risk

and low-risk groups and performed GO and KEGG enrichment analysis using the org.Hs.eg.db and clusterProfiler packages. The results showed that these DEGs were enriched in multiple cellular components, including neuronal cell body, synaptic membrane, transmembrane transporter complex, and ion channel complex. In terms of biological processes, these genes were mainly enriched in the regulation of chemical synaptic transmission, regulation of membrane potential, and trans-synaptic signaling, etc.; in terms of molecular function, activities such as monatomic and metal ion transmembrane transporter activity, voltage-gated ion channel activity, and general ion channel activity were significant, indicating that these genes are mainly involved in ion channel-related functions (Fig. 6c). In addition, KEGG pathway analysis revealed significant enrichment in neuroactive ligand-receptor interaction, pancreatic secretion, and calcium signaling-related pathways (Fig. 6d).

3.7 Somatic Mutation Analysis of Risk Score Groups

We also analyzed the genomic mutation characteristics associated with the MDAGs gene set, including mutation count and mutation spectrum. Using the Maftools package, we visualized the mutation patterns of 168 patients in the TCGA-PAAD cohort. The results showed that the overall mutation rate in the high-risk group was higher than that in the low-risk group,

(Fig-7 Genomic mutation analysis: mutation frequency and mutation patterns. (a) High-risk group. (b) Low-risk group. (c) TMB. (d) Chi-square test)

suggesting that the high-risk group may have greater genomic instability. TP53 and KRAS were the most frequently mutated genes in the high-risk group, with mutation frequencies significantly higher than those in the low-risk group (TP53: 74% vs. 48%, KRAS: 87% vs. 43%, Fig. 7a,b). Mutations in these genes are closely associated with the progression and poor prognosis of various cancers, implying that they may play a key role in disease progression in high-risk patients. In addition, CDKN2A and SMAD4 showed high mutation frequencies in both groups and were slightly higher in the high-risk group, further supporting their importance in tumor suppression and signaling.

Regarding the tumor mutation burden (TMB) of genomic mutation features, the proportion of high TMB samples in the low-risk group was higher than that in the high-risk group, and the chi-square test showed that this difference was statistically significant (Fig. 7c,d, $p = 1.7e-05$). This may suggest that the poorer response of high-risk patients to immunotherapy may be related to their potentially stronger immunogenicity.

3.8 Validation of the Seven Genes at the Single-Cell Level and Protein Expression

(Fig-8 Analysis of the expression profiles of seven genes at the single-cell level. (a) Cell cluster annotation; gene expression distribution. (b) MET. (c) CD36. (d) CKB. (e) IGF2BP3. (f) INS. (g) ALDH3A1. (h) Distribution of the corresponding proteins of the seven genes.)

To further validate the roles of the identified seven genes, we performed an in-depth analysis of the single-cell RNA sequencing data. After data preprocessing and clustering, we ultimately identified eight cell clusters and performed detailed annotation of the cell types for each cluster, annotating them as ductal epithelial cells, macrophages, fibroblasts, etc. (Fig. 8a). The results showed that MET was mainly highly expressed in ductal epithelial cells; CD36 was primarily expressed in macrophages; the expression levels of the other genes were relatively low (Fig. 8b-g). These findings further support the important roles of these genes in the occurrence and development of pancreatic cancer. To further quantify the overall expression levels of these genes in different cell types, we calculated a module score based on the above seven genes. This module score represents the overall expression level of these seven genes in single cells. The results showed that the expression level was higher in ductal epithelial cells (Fig. 8h). These findings suggest that mitochondrial dynamics may play a key role in these cell types.

3.9 Expression of the Seven Genes in Spatial and Temporal Dimensions

(Fig-9 Spatial distribution characteristics of seven genes. (a) ST deconvolution annotation (GSE155698). (b) CKB. (c) MET. (d) CD36. (e) IGF2BP3. (f) FOXM1. (g) INS. (h) ALDH3A1. Pseudotime analysis (i) and cell developmental trajectory (j). Stage-specific dynamics of (k) ALDH3A1, (l) CD36, (m) CKB, (n) FOXM1, (o) IGF2BP3, (p) INS, and (q) MET.)

Through spatial transcriptomics analysis of ST data (GSE274557), the spatial distribution characteristics of the seven core genes in pancreatic cancer tissues were studied (Fig. 9a-g). Using the single-cell annotation reference map and data from GSE155698, RCTD was employed to deconvolve the cell types of ST spots, yielding major cellular components including tumor stem cells, ductal epithelial cells, endothelial cells, fibroblasts, and macrophages (Fig. 9j). Spatial localization showed that MET and CKB genes were mainly localized in the ductal epithelial region and fibroblast region, consistent with the scRNA-seq analysis results, while the other genes exhibited a scattered low-expression pattern. Next, pseudotime trajectory analysis based on SpaCET revealed the cellular developmental trajectory (Fig. 9i, j) and stage-specific dynamics (Fig. 9k-q). MET expression gradually increased in late-stage malignant cells, whereas the INS gene showed a pseudotime-independent pattern.

Discussion

Mitochondrial dynamics refers to the dynamic balance of mitochondrial fission and fusion, which is the core mechanism for maintaining mitochondrial quantity, morphology, and functional homeostasis. Its precise regulation is key for cells to adapt to metabolic stress. In recent years, studies have found that multiple regulatory genes involved in this process exhibit obvious abnormalities in tumor cells, leading to dysregulation of the mitochondrial dynamics process, which may be closely related to the malignant process of tumors (Kang et al. 2026). The RAS-RAF-MEK-ERK signaling axis has been confirmed to directly phosphorylate the Ser616 site of DRP1 (Awad and Abdul Karim 2025), thereby promoting mitochondrial fission and fragmentation. The PINK1/Parkin pathway initiates a ubiquitination cascade when mitochondria

are damaged, marking damaged mitochondria for mitophagy (Iwata et al. 2025). These complex regulatory networks make mitochondrial dynamics likely a "central hub" (important process) for tumor metabolic reprogramming and stress adaptation. In pancreatic ductal adenocarcinoma (PDAC), abnormal mitochondrial dynamics exhibits unique biological characteristics, closely related to the metabolic plasticity and invasiveness of tumors. Mitochondria in PDAC tumor cells display a markedly fragmented phenotype (Awad and Abdul Karim 2025), and this imbalance in mitochondrial dynamics is likely closely associated with PDAC metabolic plasticity and invasiveness. Therefore, targeting this process is a highly promising therapeutic strategy in the PDAC field. It has been demonstrated that inhibiting some abnormally expressed mitochondrial dynamics-related genes can promote the restoration of normal mitochondrial morphology, thereby inhibiting PDAC tumor growth (Yu et al. 2019).

In our study, we adopted multiple machine learning methods and identified seven mitochondrial dynamics-related genes highly associated with pancreatic cancer prognosis: ALDH3A1, CD36, CKB, FOXM1, IGF2BP3, INS, and MET. Among them, ALDH3A1 is an NAD⁺-dependent enzyme that can oxidize various endogenous and exogenous aldehydes to their corresponding carboxylic acids (Estey et al. 2007). ALDH3A1 has been reported to be closely related to changes in tumor cell biological behavior, such as regulating metabolic reprogramming through the HIF-1 α /LDHA pathway, promoting cell proliferation, epithelial-mesenchymal transition (EMT), metastasis, and cancer stem cell proliferation, and inhibiting immune surveillance function (Qu et al. 2020; Terzuoli et al. 2019; Fan et al. 2021). In pancreatic cancer, studies have shown that ALDH3A1 is significantly upregulated and participates in various biological functions of tumor cells, including drug resistance, proliferation, and differentiation, especially related to fatty acid oxidation processes. It can protect mitochondrial membrane integrity by consuming toxic aldehydes, potentially providing a survival advantage in high metabolic stress high-risk tumors, and is closely related to tumor prognosis (Zhou et al. 2024; Duong et al. 2017; Weiss-Sadan et al. 2023). Its isomer ALDH1A1 is a classic cancer cell stemness marker and is significantly upregulated in pancreatic cancer. Whether ALDH3A1 shows similar performance in pancreatic cancer deserves further exploration. Meanwhile, MET (c-Met) is a proto-oncogene encoding the hepatocyte growth factor receptor (HGF-R), belonging to the tyrosine kinase receptor family, and plays an important role in biological processes such as cell proliferation, migration, invasion, and survival (Yang et al. 2022). Studies have shown that HGF/c-Met signaling participates in the tumor metastasis process by activating its downstream effector components. Specifically, Met localizes to mitochondrial fission sites and catalyzes the phosphorylation of Fis1 Tyr38, thereby promoting mitochondrial fission, which further triggers actin filament remodeling to form lamellipodia or invadopodia, playing a key role in cancer cell metastasis. Knockout of Fis1 Tyr38 can effectively prevent HGF-induced enhanced cell migration (Kermorgant and Parker 2008; Yu et al. 2021), indicating that Met-regulated mitochondrial activity is closely related to cancer cell survival. In addition, studies have shown that HGF/c-Met signaling also plays an important role in the dynamic interaction between pancreatic cancer and the stroma (Sharma and Malviya 2023). In pancreatic cancer, c-Met activation is closely associated with gemcitabine resistance (Zhu et al. 2011), tumor cell motility (Park et al. 2012), and increased angiogenic factors (Hage et al. 2013). These studies reveal the central role of c-Met signaling in multiple malignant phenotypes. CD36 is a transmembrane glycoprotein (GP), belonging to the scavenger receptor expressed in

multiple cell types, mediating various biological processes such as lipid uptake, immune recognition, inflammatory response, molecular adhesion, and apoptosis (DeFilippis et al. 2012). In tumor tissues, CD36 is expressed not only in tumor cells but also in stromal cells and immune cells, and its expression level varies depending on cell type and tumor stage (Pascual et al. 2017). Studies have shown that CD36 plays a promoting role in tumor growth and metastasis, which may be related to the enhanced lipid deposition and metabolic capacity of tumor cells, as well as lipid metabolism regulating tumor metastasis-initiating cells and the cancer epithelial-mesenchymal transition process through CD36-dependent pathways (Wang and Li 2019). Research on CD36 expression in pancreatic cancer is relatively limited, but it has been found that CD36 expression in pancreatic cancer cells and tissues is significantly lower than that in corresponding non-tumor normal tissues, and low CD36 expression is associated with lower TNM stage and CA19-9 levels, but also with larger tumor size and poorer prognosis (Jia et al. 2018). In addition, other studies have shown that CD36 expression affects gemcitabine resistance by regulating anti-apoptotic proteins, and high CD36 expression is an important adverse prognostic factor in pancreatic ductal adenocarcinoma (PDAC) patients receiving gemcitabine chemotherapy (Kubo et al. 2020). These inconsistent results still require further investigation. INS encodes insulin, which is not only one of the important endocrine hormones in the human body but also a key hormone regulating blood glucose, playing a critical role in cellular energy metabolism and survival. INS influences tumor progression through energy metabolism regulation or related pathways. In pancreatic cancer patients, especially those with hyperinsulinemia caused by insulin resistance, insulin activates the predominant IR-A receptor on pancreatic cancer cells, thereby upregulating the PI3K/AKT and MAPK/ERK pathways and the mTOR axis (Wu et al. 2023; Wang et al. 2019), and may even synergize with KRAS mutations and potentially be associated with abnormal mitochondrial dynamics, thereby promoting pancreatic cancer progression (Awad and Abdul Karim 2025; Murata et al. 2024; Gallagher and LeRoith 2020). Although the specific mechanism of INS in pancreatic cancer is not fully understood, the risk model in this study suggests it may be an important prognostic gene, and future research needs to deeply explore how INS-regulated metabolic signaling pathways specifically affect mitochondrial dynamics and chemoresistance. In summary, the seven genes in this risk model do not act in isolation, but converge on the core node of mitochondrial dynamics regulation, synergistically promoting the metabolic plasticity, invasiveness, and drug resistance of PDAC.

In this study, based on multiple machine learning algorithms, patients were divided into high- and low-risk groups by risk score, aiming to identify potential drugs with significant impact on disease prognosis. The results showed that PAAD patients with high-risk scores may be more prone to resistance to gemcitabine, oxaliplatin, cisplatin, and other chemotherapeutic drugs that interfere with DNA synthesis, as well as the topoisomerase I inhibitor irinotecan. The resistance mechanisms to drugs interfering with DNA synthesis mainly involve enhanced DNA repair capacity and altered cellular metabolic pathways; for example, enhanced mitochondrial fission can maintain dNTP pool homeostasis and provide nucleotide precursors, promoting DNA repair, while reactive oxygen species generated due to mitochondrial dysfunction can induce DNA damage, reversely activating repair mechanisms (Srinivas et al. 2019; Fang et al. 2016). In addition, enhanced DNA repair capacity can repair DNA damage caused by topoisomerase I inhibitors, thereby reducing the killing effect of irinotecan (Maede et al. 2014; Alagoz et al. 2012; Stoop et al. 2024).

This also potentially relates to our finding that the high-risk group is enriched in DNA repair pathways. Furthermore, we found that patients in the high-risk group may be more sensitive to multiple targeted drugs, including MEK inhibitors (Selumetinib, Trametinib), various tyrosine kinase inhibitors (Ulixertinib, Osimertinib, Lapatinib, Gefitinib, Dasatinib), and Acetalax (whose mechanism differs from tyrosine kinase inhibitors) (Kenney et al. 2021; Ko et al. 2016; Zhang et al. 2025; Grierson et al. 2023; Vigié et al. 2025; Striefler et al. 2022; Pino et al. 2006; Morton et al. 2010; Morrison et al. 2013), suggesting that these drugs may be potential therapeutic options for pancreatic cancer patients. The related signaling pathways deserve further investigation to evaluate the feasibility of targeting strategies.

The TME (tumor microenvironment) plays a critical role in the occurrence and spread of pancreatic cancer. The tumor microenvironment is composed of various cell types closely related to tumor initiation, proliferation, and migration, including immune cells, stromal cells, endothelial cells, a complex cytokine secretion network, and fibroblast remodeling, among other components (Hinshaw and Shevde 2019). Moreover, the tumor immunosuppressive microenvironment can also limit the clinical efficacy of tumor immunotherapy, especially immune checkpoint blockade (ICB) and cell therapy, casting doubt on the prospects of this treatment modality. In this study, the immune infiltration results showed that the infiltration of immune inflammatory cells in the low-risk group was significantly higher than that in the high-risk group, including activated B cells, eosinophils, macrophages, mast cells, and monocytes. This is consistent with the description of the pancreatic cancer immune microenvironment in existing literature. One of the most prominent features of the PDAC TME is the lack of infiltration of cytotoxic CD8 + T cells (Daley et al. 2016). The main barrier to the anti-tumor adaptive immune response in PDAC appears to be insufficient antigen presentation. Additionally, the number of dendritic cells (DCs) in PDAC is low (Hegde et al. 2020), and various factors lead to the characteristic of pancreatic cancer as an immune "cold" tumor (Barriga et al. 2022). The assessment of immune function showed that we observed significantly enhanced cytolytic activity, T cell co-stimulation response, and type II interferon response in the low-risk group, exhibiting "hot tumor" characteristics, suggesting that these patients may have stronger anti-tumor immune responses. This is consistent with the immune cell infiltration results, indicating that the tumor microenvironment of the low-risk group may be more conducive to the immune system's response, thereby inhibiting tumor progression. This study also showed that in patients with high-risk scores, the expression levels of multiple immune checkpoint genes such as BTLA, CD276, CD44, HHLA2, LGALS9, CD160, TNFSF15, and TNFSF9 were high, indicating that these genes may become potential targets for targeted immunotherapy in the future. However, the predictive analysis of ICB treatment showed no significant difference in response to ICB therapy between the high- and low-risk groups, which may be related to the insensitivity of pancreatic cancer to immunotherapy as a "cold tumor". Therefore, immunotherapy strategies for pancreatic cancer still require in-depth exploration, especially synergistic strategies involving mitochondrial dynamics regulation and immune checkpoint blockade, in order to overcome resistance mechanisms.

In the study of somatic mutations, we found that the mutation frequencies of TP53 and KRAS were significantly higher in the high-risk group than in the low-risk group. In addition, CDKN2A and SMAD4 also showed high mutation frequencies in both groups. Extensive studies have shown that mutations in KRAS

and TP53 profoundly affect the survival of pancreatic cancer patients. KRAS, as a proto-oncogene, leads to the sustained activation of the RAS/RAF/MEK/ERK signaling pathway when mutated, thereby promoting cell proliferation, survival, and migration (Mondal et al. 2024). TP53, as a tumor suppressor gene, usually causes loss of cell cycle control and reduced DNA repair capacity when mutated, promoting tumor development (Negrini et al. 2010). CDKN2A mutations usually lead to abnormal cell cycle regulation, while SMAD4 mutations or deletions are closely related to pancreatic cancer metastasis (Hu et al. 2018).

In addition, we analyzed the single-cell sequencing data of the seven genes included in the model and observed that at the single-cell level, the distribution of cells positive for these molecules in pancreatic cancer was different. Notably, these cells were mainly distributed in ductal epithelial cells, acinar cells, and some tumor stem cells; for example, the MET gene was highly expressed in ductal epithelial cells. Further spatial transcriptomics analysis of these seven genes using ST data showed a distribution consistent with the above features, suggesting that mitochondrial dynamics-related processes may be active in these cell types. There may be an association between TP53 and KRAS mutations and the above seven genes, but the direction and mechanism still need further validation. Perhaps, in pancreatic cancer cells, in addition to the reported mitochondrial dynamics-related pathways, there may be other regulatory mechanisms worth further investigation. Finally, we performed pseudotime analysis using the ST dataset and found that the expression levels of these seven genes varied during the differentiation and development of pancreatic cancer, with some genes (such as MET) gradually increasing in expression with pseudotime stage in malignant tumors. This may be related to the activity and role of mitochondrial dynamics at various stages in pancreatic tumor cells, which warrants more in-depth exploration and discussion.

Overall, we conducted a systematic analysis of mitochondrial dynamics in PAAD. First, we revealed the abnormal mitochondrial dynamics-related expression profile in PAAD, suggesting that abnormal expression of mitochondrial dynamics-related genes may be associated with PAAD progression. Second, we constructed a prognostic model related to mitochondrial dynamics and found that ALDH3A1, CD36, CKB, FOXM1, IGF2BP3, INS, and MET showed high sensitivity and specificity in the diagnosis and prognosis of PAAD patients. In addition, we found that these CARGs features are closely related to malignant clinical manifestations, clinicopathological indicators, immunosuppressive tumor microenvironment, and prediction of chemotherapy drug sensitivity. We also introduced spatial transcriptomic data to explore the relationship between the spatiotemporal distribution of malignant tumors and the expression of the above seven genes, and finally used pseudotime analysis to predict the changes in the expression of these seven key genes during the differentiation and development of pancreatic cancer.

However, this study still has some limitations. First, this study was primarily a retrospective analysis based on public databases, and differences in sample sources, sequencing platforms, and data processing pipelines may introduce batch effects and selection bias. Second, although the risk model was validated in external cohorts, it still lacks prospective clinical cohort validation, and its clinical applicability requires further evaluation. Third, drug sensitivity and immunotherapy response analyses are mainly based on computational predictions and cannot be directly equated to actual patient efficacy. Finally, this study has not yet verified the specific mechanisms by which the seven core genes regulate mitochondrial dynamics

and affect pancreatic cancer progression through in vivo and in vitro experiments, and further confirmation in combination with functional experiments and clinical samples is needed in the future.

In conclusion, the mitochondrial dynamics signature in this study provides a powerful new tool for prognostic assessment and precision treatment of pancreatic cancer. It reveals how upstream signaling pathway perturbations converge and reprogram mitochondrial dynamics, thereby promoting malignant phenotypes, DNA damage repair, and immune evasion of tumors. Future research should focus on experimentally validating the specific molecular mechanisms by which these genes regulate mitochondrial dynamics in pancreatic cancer models, testing the clinical utility of this model in prospective cohorts, and exploring combination therapeutic strategies targeting mitochondrial dynamics.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Conceptualization: Ma Gongping.

Writing – original draft: Ma Gongping.

Writing – review and editing: Liu Yuchuan.

Project administration: Qin Shu.

Resources: Qin Shu.

Data Availability

No new datasets were generated during this study. All datasets analysed are publicly available from the repositories listed below. The relevant cohort identifiers and accession numbers are provided to support reproducibility.

TCGA pancreatic adenocarcinoma cohort (TCGA-PAAD), NCI Genomic Data Commons:
<https://portal.gdc.cancer.gov/projects/TCGA-PAAD>

Normal pancreatic tissue data, Genotype-Tissue Expression (GTEx) Portal: <https://gtexportal.org/home/>

ICGC pancreatic cancer cohort (PACA-AU), International Cancer Genome Consortium:

<https://dcc.icgc.org/projects/PACA-AU>

NCBI Gene Expression Omnibus datasets: GSE78229; GSE57495; GSE62452; GSE155698; GSE274557

Protein-expression and immunohistochemistry data, Human Protein Atlas: <https://www.proteinatlas.org/>

Immunotherapy-related data, The Cancer Immunome Atlas: <https://tcia.at/home>

Mitochondrial dynamics-related gene information, GeneCards: <https://www.genecards.org/>

Ethics Approval

This study used only de-identified data obtained from publicly accessible databases and did not involve direct participant recruitment, intervention, or the collection of new human biological samples. The Research Ethics Committee of The Affiliated Hospital of Southwest Medical University confirmed that ethical approval was not required for this study. The requirement for informed consent was therefore not applicable.

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Figures

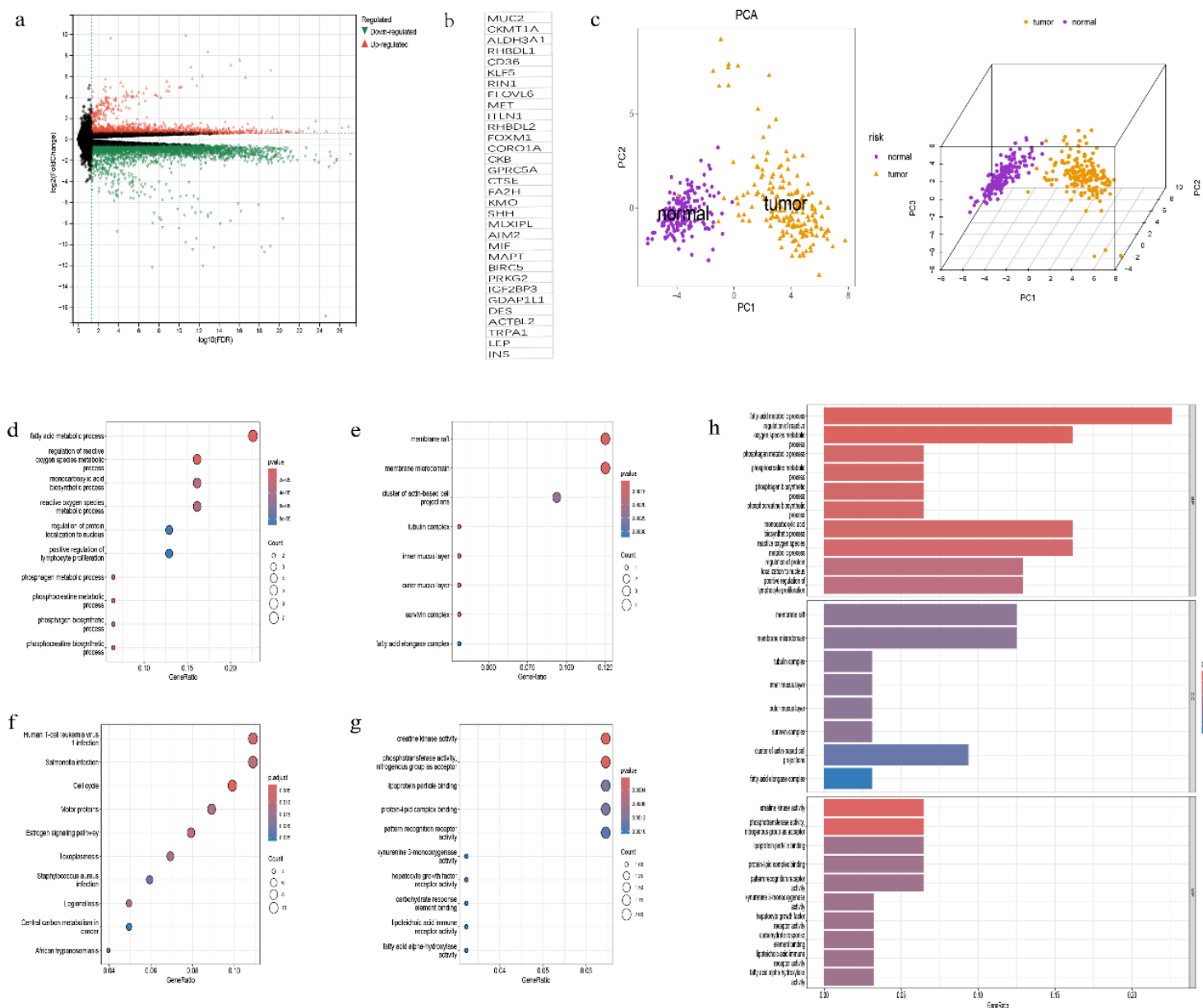


Figure 1

Differential gene analysis and enrichment analysis of PAAD samples using TCGA combined with GTEx.
 a) DEGs in PAAD tumors (b) 32 differentially expressed key mitochondria dynamics-related genes (c) Principal component analysis (PCA) of the 32 differential genes (d) Biological process annotation of the 32 DEGs (e) Cellular component annotation of the 32 DEGs (f) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment of the 32 DEGs (g) Molecular function annotation of the 32 DEGs (h) Gene Ontology (GO) functional annotation of the 32 DEGs

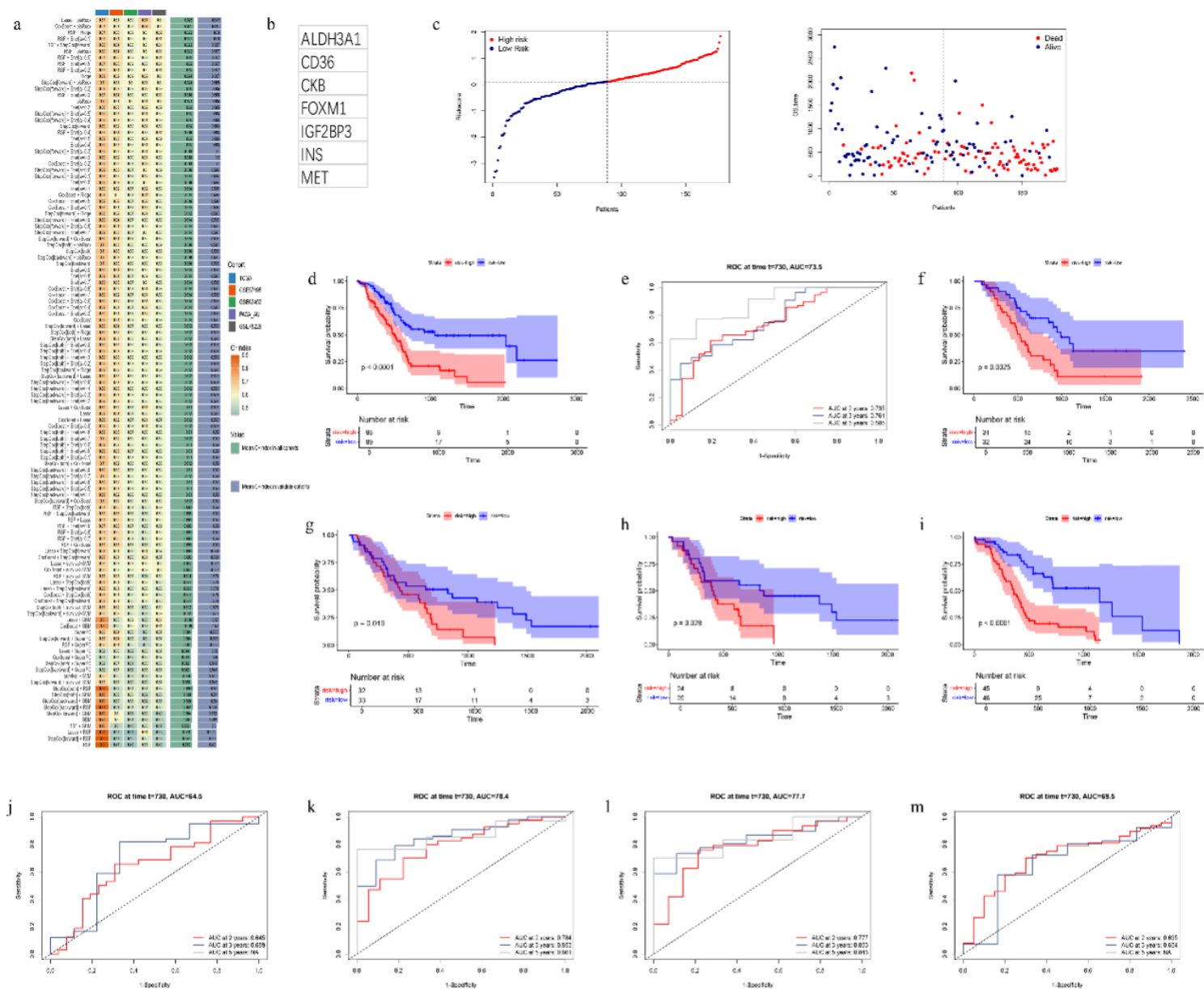


Figure 2

Machine learning-based risk prediction model set, scatter plots, survival curves, and ROC curves. (a) Risk prediction model set; (b) Seven genes with significant impact on survival outcomes; (c), Scatter plot; (d,e) TCGA-PAAD; (f,j) GSE57495; (g,k) GSE62452; (h,l) GSE78229; (i,m) ICGC

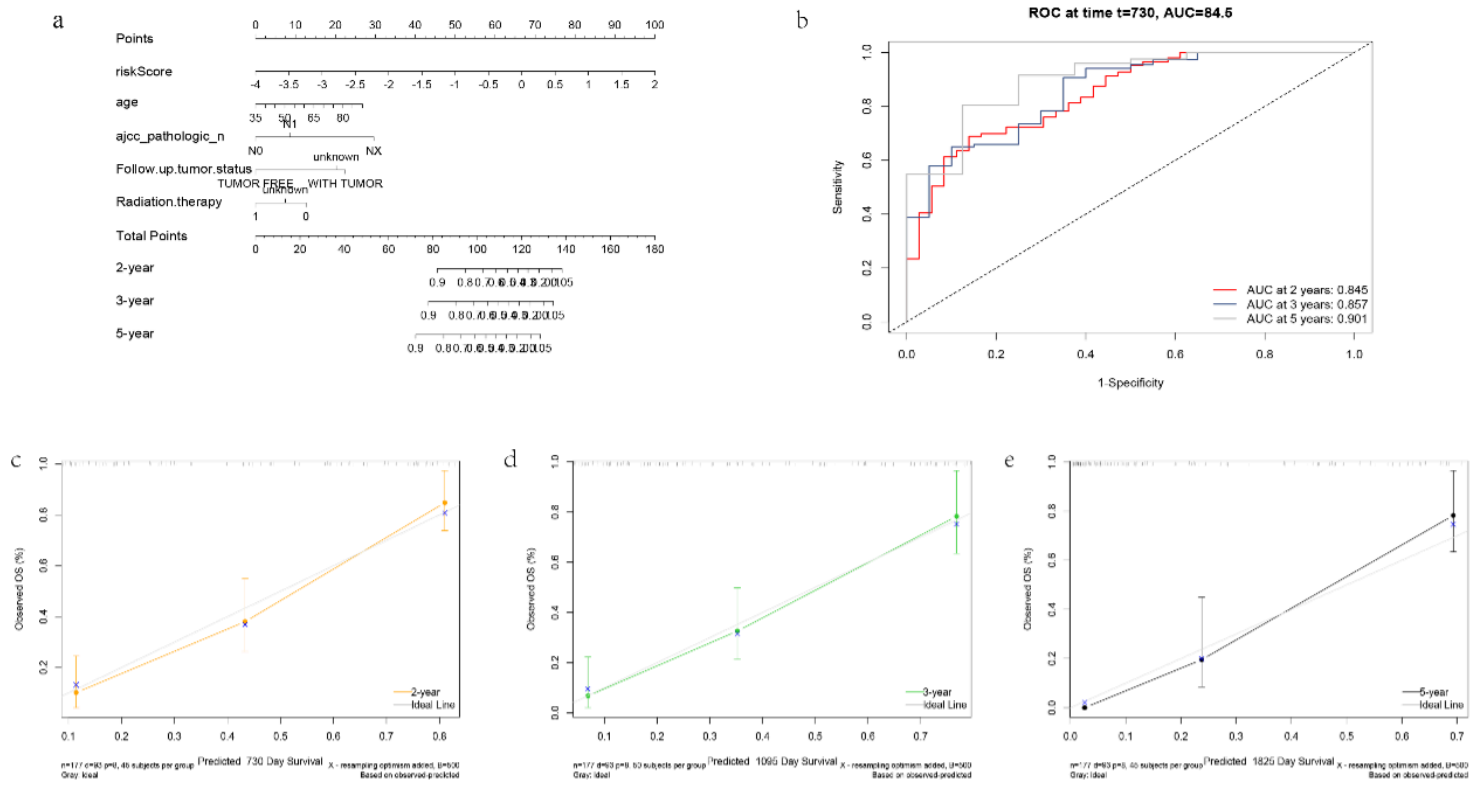


Figure 3

Validation of independent prognostic factors of the mitochondrial dynamics-related risk score. (a) Stepwise Cox regression and nomogram. (b) AUC values for predicting 2-, 3-, and 5-year overall survival. Calibration curves for (c) 2-year, (d) 3-year, and (e) 5-year survival

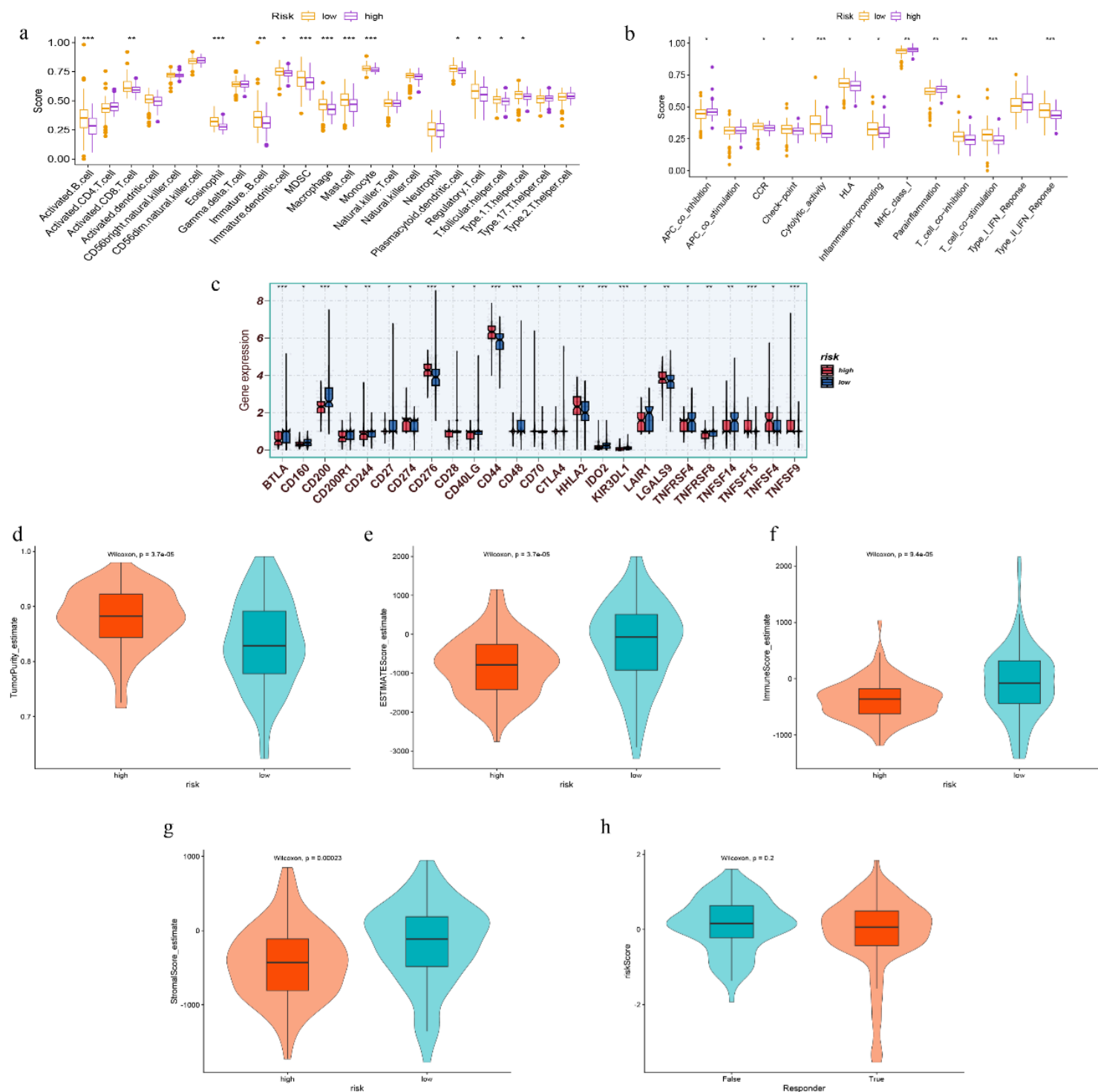


Figure 4

Immune infiltration and tumor microenvironment. (a) Immune cell infiltration (ssGSEA). (b) Immune function. (c) Immune checkpoint. (d) Tumor purity. (e) ESTIMATE score. (f) Immune score. (g) Stromal score. (h) TIDE score.

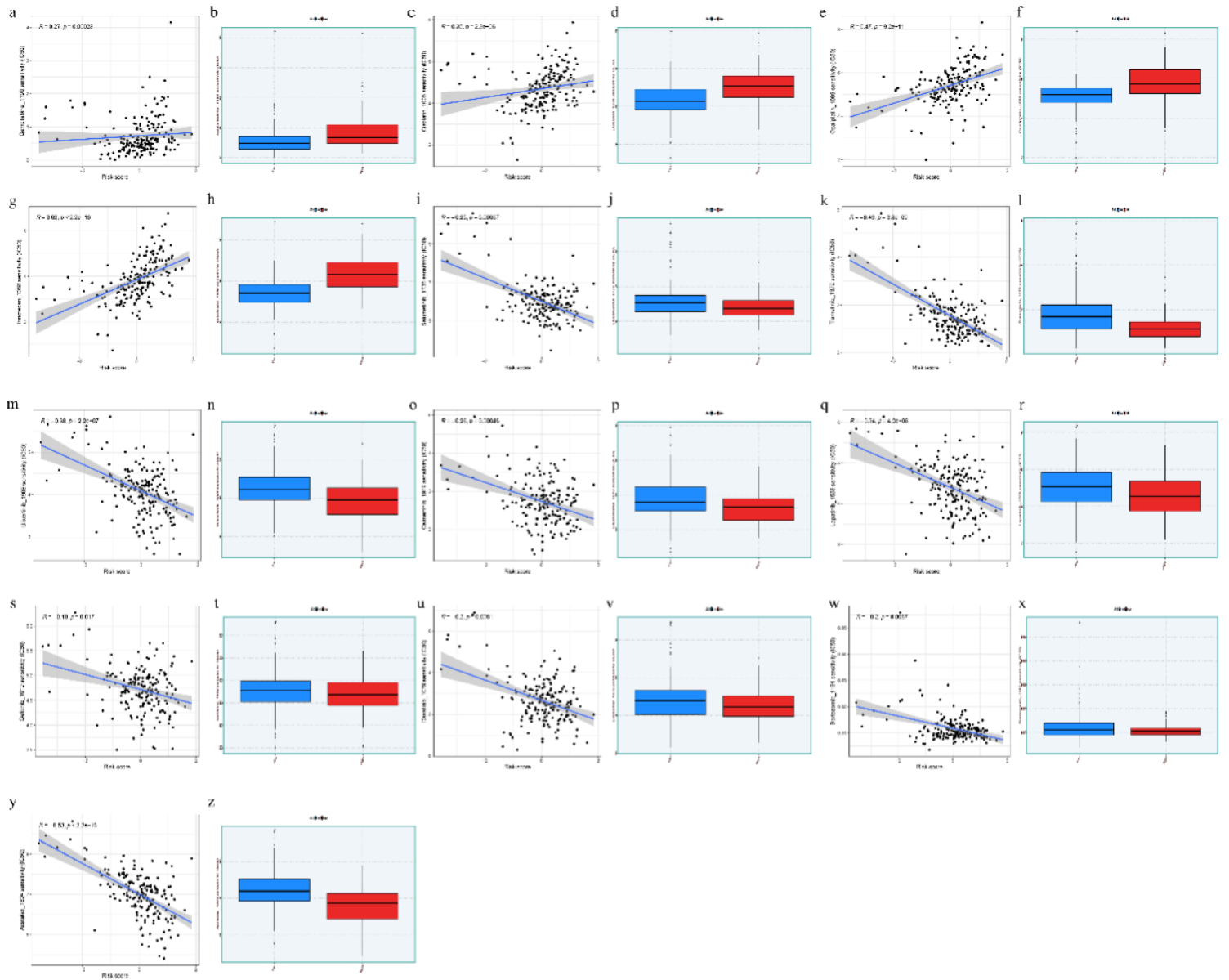


Figure 5

Prediction of chemotherapy drug response. Correlation between risk score and efficacy; prediction of drug response (IC50) in high- and low-risk groups. (a,b) Gemcitabine. (c,d) Cisplatin. (e,f) Oxaliplatin. (g,h) Irinotecan. (i,j) Selumetinib. (k,l) Trametinib. (m,n) Ulixertinib. (o,p) Osimertinib. (q,r) Lapatinib. (s,t) Gefitinib. (u,v) Dasatinib. (w,x) Bortezomib. (y,z) Acetalax.

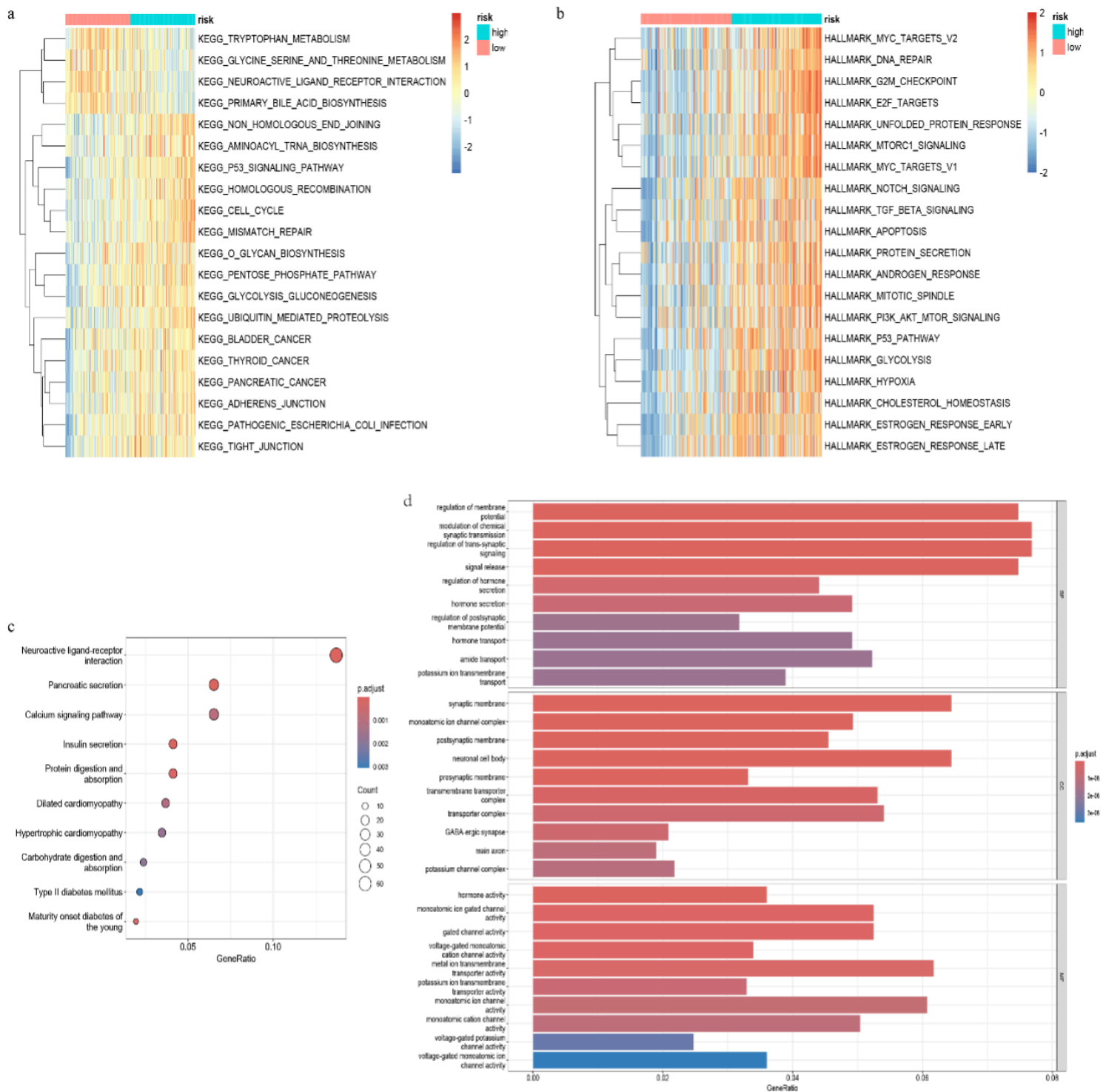


Figure 6

Functional annotation analysis of mitochondrial dynamics-related gene sets. (a) GSVA-KEGG heatmap. (b) GSVA-HALLMARK enrichment analysis. (c) GO. (d) KEGG.

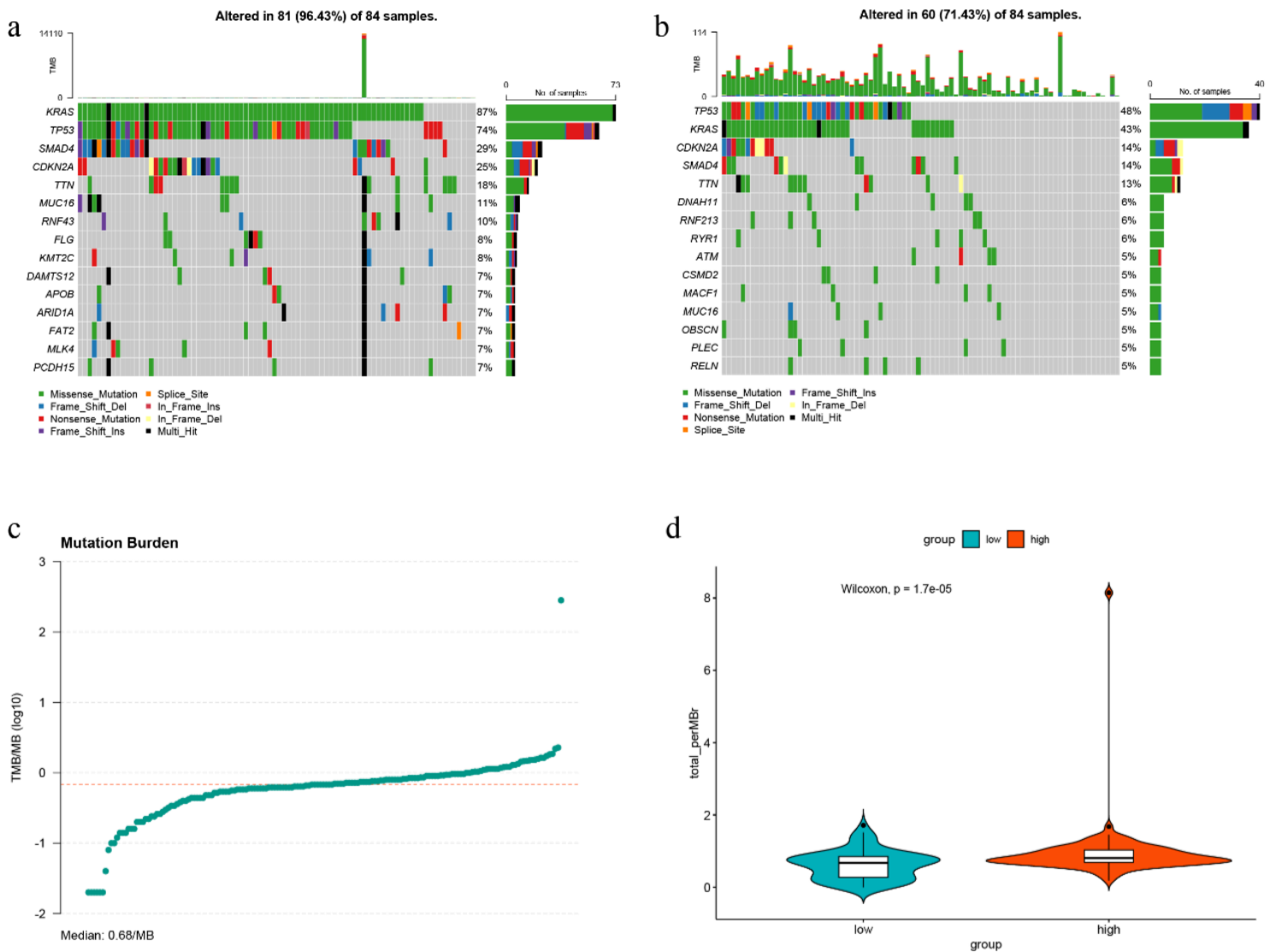


Figure 7

Genomic mutation analysis: mutation frequency and mutation patterns. (a) High-risk group. (b) Low-risk group. (c) TMB. (d) Chi-square test

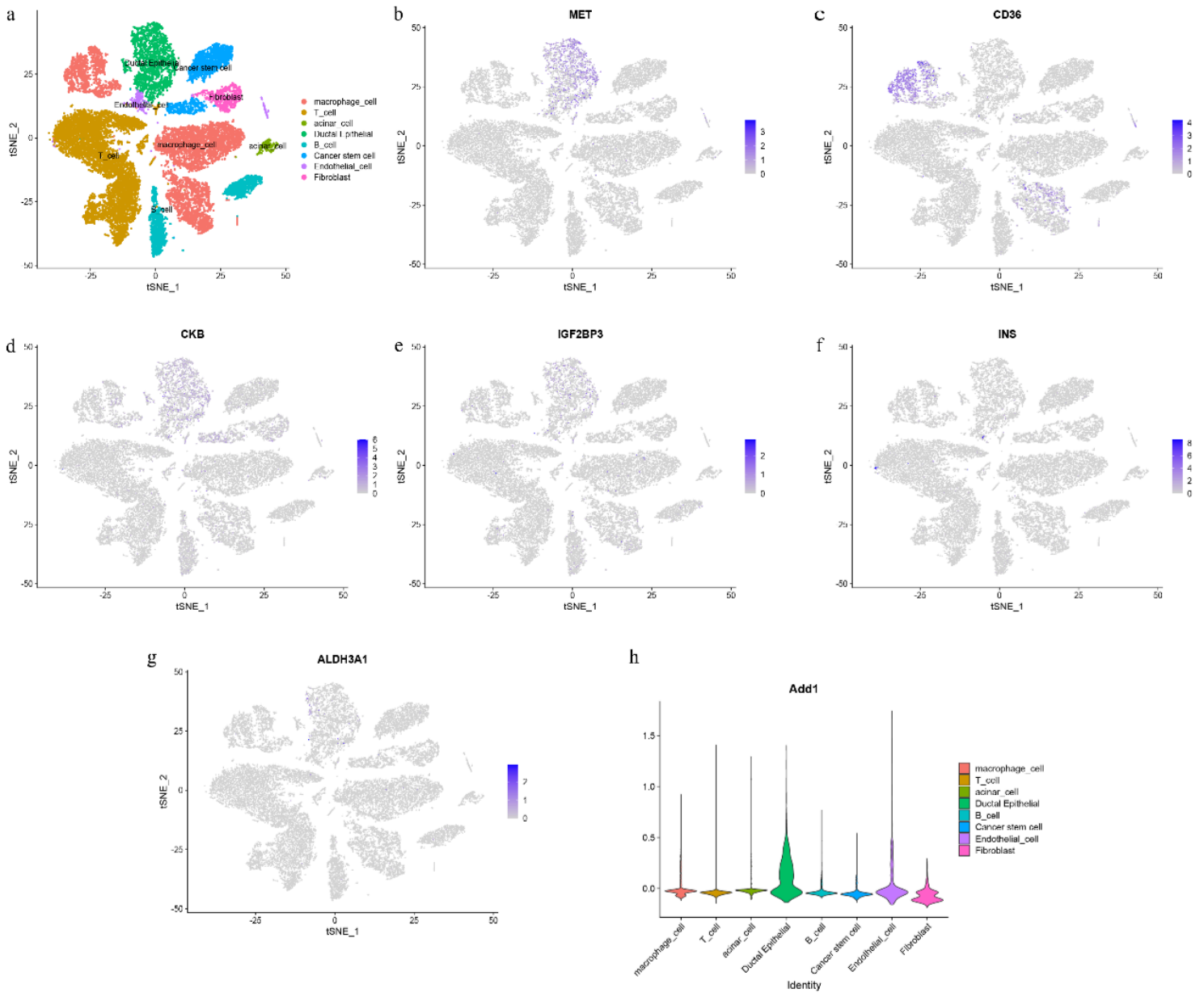


Figure 8

Analysis of the expression profiles of seven genes at the single-cell level. (a) Cell cluster annotation; gene expression distribution. (b) MET. (c) CD36. (d) CKB. (e) IGF2BP3. (f) INS. (g) ALDH3A1. (h) Distribution of the corresponding proteins of the seven genes.

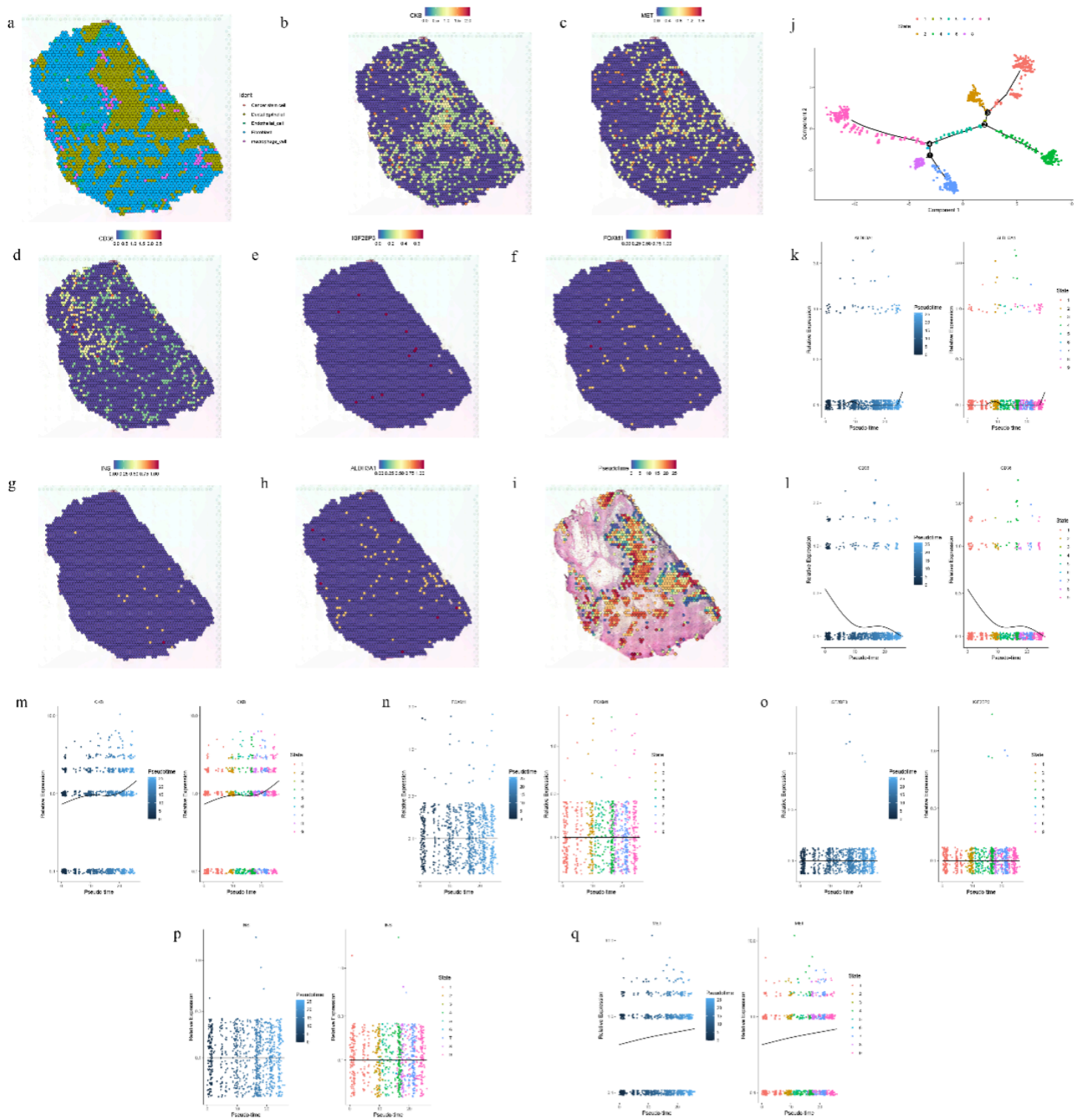


Figure 9

Spatial distribution characteristics of seven genes. (a) ST deconvolution annotation (GSE155698). (b) CKB. (c) MET. (d) CD36. (e) IGF2BP3. (f) FOXM1. (g) INS. (h) ALDH3A1. Pseudotime analysis (i) and cell developmental trajectory (j). Stage-specific dynamics of (k) ALDH3A1, (l) CD36, (m) CKB, (n) FOXM1, (o) IGF2BP3, (p) INS, and (q) MET.