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Development of a KRAS-Associated Metabolic Risk Model for Prognostic Prediction in Pancreatic Cancer

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Abstract

Background: KRAS was reported to affect some metabolic genes and promote metabolic reprogramming in solid tumors. However, there is no comprehensive analysis to explore KRAS associated metabolic signature or risk model for Pancreatic cancer (PC).

Methods: In current study, multiple bioinformatics analyses were used to identify differentially expressed metabolic genes based on KRAS mutation status in PC. Then we developed and validated a prognostic risk model based on the selected KRAS-associated metabolic genes. Besides, we explored the association of the risk model and the metabolic characteristics as well as Gemcitabine associated chemoresistance in PC.

Results: 6 KRAS-associated metabolic genes (i.e. CYP2S1, GPX3, FTCD, ENPP2, UGT1A10, and XDH) were selected and were enrolled to establish a prognostic risk model. The prognostic model had a high C-index of 0.733 for overall survival (OS) in the TCGA pancreatic cancer database. The area under the curve (AUC) values of 1- and 3-year survival were both greater than 0.70. Then the risk model was validated in two GEO datasets and also presented a satisfactory discrimination and calibration performance. Further, we found that the expression of some KRAS-driven glycolysis associated genes (PKM, GLUT1, HK2, and LDHA) and Gemcitabine associated chemoresistance genes (i.e. CDA and RMM2) were significantly up-regulated in high-risk PC patients evaluated by the risk model.

Conclusions: We constructed a risk model based on 6 KRAS associated metabolic genes, which predicts patients' survival with high accuracy and reflects tumor metabolic characteristics and Gemcitabine associated chemoresistance in PC.

Introduction

Pancreatic cancer (PC) is one of the most malignant cancers, which leads to 4.5% of all cancer-related deaths globally.[1] The vital causes for the poor prognosis are the highly aggressive phenotype and early cancer recurrence and metastasis following surgical treatment.[2] Despite some advances in the management of PC in recent years, very few major breakthroughs for effective biomarkers nor treatment strategies have emerged.

Almost all PC patients carry at least one of the four frequently-mutated driver genes, which are oncogene KRAS and the tumor suppressors TP53, SMAD4 and CDKN2A.[3] KRAS, the most frequently mutated oncogene in cancer especially in PC, was reported to rewire metabolism to support tumor growth.[4] Several studies had shown that KRAS promoted metabolic reprogramming through the enhance of glucose metabolism, differential channeling of glucose intermediates, reprogramming glutamine metabolism, increasing autophagy, and macropinocytosis.[5, 6] The metabolic reprogramming and fibrotic stroma of PC had been considered as a barrier for cytotoxic drugs delivery to cancer cells, therefore contributing to chemoresistance and treatment failure.[7, 8] Although some KRAS-associated genes were found to regulate cancer cell metabolism, the mechanism was still remain to be clarified and

there was no comprehensive analysis to explore KRAS associated metabolic signature or risk model for PC.

In current study, multiple bioinformatics analyses were used to identify differentially expressed metabolic genes based on KRAS mutation status in PC. Then a prognostic model was constructed based on the selected KRAS-associated metabolic genes. Moreover, we explored the association of the risk model and the metabolic characteristics along with the Gemcitabine associated chemoresistance in PC.

Materials And Methods

Datasets and data acquisition

The gene expression data recorded based on Fragments Per Kilobase per Million (FPKM) and clinical information for 178 PC samples were obtained from the Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/repository) database up to September 2020. The KRAS mutation data of TCGA PC cohort were downloaded from cBioportal (http://www.cbioportal.org/).[9] Among these 178 PC samples, 167 PC samples with RNA-sequencing data and KRAS mutation information were subjected to subsequent analysis. GSE57495 data set based on GPL9115 (including 63 PC samples) and GSE79668 data set based on GPL11154 (including 51 PC samples) were downloaded from Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database for validation. Besides, two other GEO datasets, GSE140077 based on GPL20795 (including 1 PC cell lines and 6 samples) and GSE106336 based on GPL18573 (including 1 PC cell line and 6 samples) were used for further study in Gemcitabine resistance of PC. A total of 1724 metabolic genes were obtained from the metabolic pathway-related gene lists of “c2.cp.kegg.v7.1.symbols.gmt" in gene set enrichment analysis (GSEA, https://www.gsea-msigdb.org/gsea/index.jsp).[10] Datasets above for PC are publicly obtainable and ethics approval was not needed.

Differential expression analysis

We compared 58 PC samples without KRAS mutations (KRAS WT) and 109 PC samples with KRAS mutations (KRAS MUT) in TCGA PC cohort to identify differentially expressed metabolic genes (DEGs) using the R package limma. The thresholds were |log2-fold change (FC)|>1.5 and FDR<0.05.

Establishment and validation of the KRAS-associated metabolic risk score system

With the help of R packages survival, survminer, and parallel, the univariate Cox proportional regression was performed to evaluate the association between expression of DEGs and patients’ overall survival (OS) in TCGA cohort. DEGs with p-value < 0.01 were considered as statistically significant prognosis-associated DEGs (PAGs). Then, a multivariate Cox proportional regression model was established

through cycle computation to determine the model with highest discriminated ability for OS of PC. The KRAS-associated metabolic risk model was established based on a linear combination of the expression values of the PAGs and the multivariate Cox regression coefficients were used as the weight. For the external validation cohort for the risk score system, the GSE57495 and GSE79668 datasets were analyzed. Patients in separate datasets were divided into a high-risk group and a low-risk group using the median cut-off of the risk score. To evaluate the performance of model, the R packages pROC were used to evaluate the discrimination of the risk model in TCGA and GEO datasets through area under the curve (AUC) of the receiver operating characteristic (ROC) curve of 1- and 3-year survival. With the help of R package rms, Harrell's concordance index (C-index) of the risk score system was calculated by a bootstrap approach with 1000 resamples. The calibration curves were used to assess the consistency between model-predicted and observed survival. Then the log-rank tests and Kaplan-Meier (KM) analyses were performed using the survival R package between the high-risk and low-risk group to assess the predictive ability of the prognostic model. We calculated the risk scores in different groups based on clinicopathological parameters (such as American Joint Committee on Cancer (AJCC) stage, histologic grade, diabetes status) in TCGA PC cohort by using Wilcoxon's test to evaluate whether the risk model reflects PC progression.

Functional enrichment analysis

Gene set enrichment analyses (GSEA, Version3.0, http://software.broadinstitude.org/gsea/) was performed to determine how the metabolic pathways and relevant metabolic pathway-related genes differed between PC samples of KRAS WT and KRAS MUT in TCGA cohort. An annotated gene set file (c2.cp.kegg.v7.1.symbols.gmt) was used as the reference gene set and a gene set was considered as an enriched group when nominal p-value < 0.05. DEGs and co-expressed genes of PAGs screened from cBioPortal database were integrated to DAVID 6.7 (https://david-d.ncifcrf.gov/) separately to perform Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.[11] Results were visualized by using R package ggplot2.

Association between the risk model with metabolic characteristics of PC

Several KRAS-driven metabolic targets which took part in metabolic reprogramming of PC were selected to explore relationships between the risk model with metabolic characteristics of PC.[12] The mRNA expressions levels of these promising KRA-driven metabolic genes were compared in high-risk and low-risk groups by using Student's t-test.

Association between the risk model and Gemcitabine chemoresistance of PC

In order to evaluate whether the risk model reflects Gemcitabine metabolism-associated chemoresistance of PC, metabolic risk scores were calculated in 2 parental and GEM-resistant cell lines (CFPAC-1 and HPAFII) in GSE140077 and GSE106336 datasets. Then several Gemcitabine metabolism-associated chemoresistance genes of PC were selected to explore relationships between the risk model with Gemcitabine chemoresistance of PC.[13] The mRNA expressions levels of these promising KRAS-driven metabolic genes were compared in high-risk and low-risk groups by using Wilcoxon's test.

Statistical analyses

All statistical analyses were performed using R software Version 4.0.1 (https://www.r-project.org/) and SPSS software Version 24.0 (SPSS, Inc., Chicago, IL, USA). If not specified above, a p-value < 0.05 was considered statistically significant.

Results

Association between metabolic phenotype and KRAS mutations in PC

It’s shown in FireBrowse Database (http://www.firebrowse.org/) that KRAS mutation was the most common type of mutation in PC, followed by mutation frequency of TP53, MAMLD1, MAGEC1, CDKN2A, and SMAD4 (Figure 1A). Next, we utilized mRNA expression data and corresponding clinical information of PC samples in TCGA and cBioportal to investigate metabolic processes linked to KRAS mutation status. GSEA was conducted to determine the difference of metabolic pathways between 58 PC samples of KRAS WT and 109 PC samples of KRAS MUT. The results showed that 4 metabolic biological processes were significantly enriched in KRAS MUT group, which were Glycine Serine and Threonine Metabolism (NES=1.63, size=31 and p-value<0.05), Tryptophan Metabolism (NES=1.48, size=40 and p-value<0.05), Type II Diabetes Mellitus (NES=1.41, size=47 and p-value<0.05), and Primary Bile Acid Biosynthesis (NES=1.67, size=16 and p-value<0.05) (Figure 1B).

Identification and enrichment analysis of differentially expressed metabolic genes based on KRAS mutation status

We performed differential expression analyses between 58 PC samples of KRAS WT with 109 PC samples of KRAS MUT in TCGA PC cohort. Among 1724 metabolic genes, 54 genes were significant differentially expressed and considered as DEGs (|log2-fold change (FC)|>1.5 and FDR<0.05) (Figure 2A, B). Then these 54 DEGs were integrated to DAVID 6.7 to perform GO analysis and KEGG pathway analysis. GO analysis showed that the top 10 highly enriched functions in the metabolic process were “Oxidation Reduction, Carboxylic Acid Catabolic Process, Hormone Metabolic Process, Alcohol Catabolic Process, Glucose Metabolic Process, Lipid Catabolic Process, Cellular Amino Acid Catabolic Process,

Vitamin Metabolic Process, Steroid Metabolic Process, and Glutamine Family Amino Acid Metabolic Process" (Figure 2C). In KEGG analysis (Figure 2D), DEGs was found to be mainly enriched in "Adipocytokine Signaling Pathway, Retinol Metabolism, Metabolism of Xenobiotics by Cytochrome P450, Type II Diabetes Mellitus, Drug Metabolism, Endocytosis, and Fatty Acid Metabolism".

Construction and validation of a KRAS-associated metabolic risk model

We performed univariate Cox proportional regression and found that 21 out of 54 DEGs were significantly related to OS and considered as prognosis-associated DEGs (PAGs) (Figure 3A). Further, with the help of the R packages survival, a multivariate Cox regression analysis for these 21 PAGs was performed and it revealed that 6 of them were independently associated with OS (Figure 3B). Therefore, a 6-PAGs based KRAS-associated metabolic risk model was developed by weighting the normalized expression of these 6 PAGs multiplied by corresponding coefficients derived from the multivariate Cox regression analysis: risk score = (-0.29328 * normalized expression of CYP2S1) + (-0.45614 * normalized expression of GPX3) + (-0.57665 * normalized expression of FTCD) + (0.54899 * normalized expression of ENPP2) + (0.19472 * normalized expression of UGT1A10) + (0.34727 * normalized expression of XDH) (Table 1). Patients were divided into high-risk and low-risk groups by using the median cut-off value of the risk score (Figure 3C).

Table 1
6 KRAS-associated metabolic genes to establish the risk model

Genes	coef	HR	HR.95L	HR.95H	P-value
CYP2S1	-0.29328	0.74582	0.60422	0.92060	0.00633
GPX3	-0.45614	0.63372	0.47754	0.84098	0.00158
FTCD	-0.57665	0.56178	0.44136	0.71504	2.80E-06
ENPP2	0.54899	1.73150	1.2754	2.3506	0.000432
UGT1A10	0.19472	1.21498	1.0496	1.4064	0.00911
XDH	0.34727	1.41520	1.1314	1.7702	0.00236

The predictive accuracy of the risk model was further assessed through the ROC and C-index analysis. The results showed the AUC of the risk model for OS in TCGA cohort was 0.773 at 1 years and 0.704 at 3 years (Figure 4A). Besides, C-index of the risk model in TCGA cohort was 0.733 (95% CI, 0.675 - 0.761). The calibration curves of the risk model matched well, which indicated that it could accurately predict the 1- and 3-year OS in TCGA cohort (Figure 4B). GSE57495 and GSE79668 datasets were used for the external validation for the risk model. The AUC for 1-, and 3-year OS in GSE57495 datasets were 0.627, and 0.698 (Figure 4A), while the AUC for 1-, and 3-year OS are 0.727 and 0.617 in GSE79668 datasets (Figure 4E). The C-index of the risk model in GSE57495 and GSE79668 datasets were 0.683 (95% CI,

0.655 - 0.723) and 0.625 (95% CI, 0.598 - 0.675). And the corresponding 1-year and 3-year calibration curves are respectively shown in Figure 4D and 4F.

Association between the risk model with patients' survival and clinicopathological characteristics in PC

To further evaluate the prognostic power of the risk model, Kaplan-Meier analyses were performed and we found that all patients in high-risk group had a shorter OS (p-valve=4.234e-07) and Disease-free survival (DFS) (p-valve=1.83e-06) than those in low-risk group in TCGA cohort (Figure 5A-B). Besides, similar results for KM analyses of OS were observed in GSE57495 dataset (p-value=8.637e-04) and GSE79668 dataset (p-valve=1.833e-02) (Figure 5C-D). Further we calculated the risk scores in different groups based on clinicopathological characteristics in TCGA PC cohort and the results showed that patients who had advanced stage, higher histologic grade or diabetes history had higher risk scores (p-valve<0.05) (Figure 5E). The data in Figure 4 and Figure 5 suggest the KRAS associated metabolic risk model has effective value in predicting patients' survival and is associated with advanced tumor characteristics.

Association between the risk model with metabolic characteristics of PC

We next explored the activities of 6 PAGs (i.e. CYP2S1, GPX3, FTCD, ENPP2, UGT1A10, and XDH) by analyzing its potential metabolic pathways in PC. The co-expression analyses for 6 PAGs were performed by using cBioPortal dataset (Spearman's correlated coefficient>0.6 or <−0.6, p-valve<0.05). We found 131 co-expression genes for CYP2S1, 163 co-expression genes for ENPP2, 387 co-expression genes for FTCD, 521 co-expression genes for GPX3, 189 co-expression genes for UGT1A10, and 213 co-expression genes for XDH, all of which were enrolled into DAVID 6.7 and subjected to functional and pathway enrichment analyses.

The potential metabolic pathways involved were shown in Figure 6A. CYP2S1 and its neighboring genes were mainly enriched in "Oxidation-reduction process, Lipid metabolic process, Protein glycosylation, Lysophospholipase activity, Steroid metabolic process, Regulation of glucuronidation, and Response to insulin stimulus". GPX3 may act a vital role in "Lipid metabolic process, Glutamate receptor activity, and Regulation of insulin secretion". FTCD may play an important role in "Glucose metabolic process and Regulation of insulin secretion". ENPP2 may be involved in "L-amino acid transport, Glutamate receptor activity, Regulation of insulin secretion, and Response to insulin stimulus". UGT1A10 was mainly associated with "Lipid metabolic process, Steroid metabolic process, Regulation of glucuronidation, CoA succinyl transferase activity, Endocytosis" and XDH was involved in "Protein glycosylation, Phagocytosis, and Endocytosis".

To further explore the potential metabolic targets influenced by our risk model, we compared the expression levels of several KRAS-driven metabolic genes between high-risk with low-risk group in TCGA cohort. We found higher mRNA expressions of PKM (p=1.86e-05), GLUT1 (p=3.168e-05), HK2 (p=3.056e-04), LDHA (p=2.989e-06), and VDR (p=0.012) in the high-risk group (Figure 6B).

Association between the risk model with Gemcitabine chemoresistance in PC

We found that 5 of 6 PAGs were enriched in “Drug metabolism or Response to drug” in Functional and pathway enrichment analyses (Figure 7A). In order to explore their relationship with Gemcitabine associated chemoresistance in PC, we calculated metabolic risk scores in 2 parental and Gemcitabine-resistant cell lines (CFPAC-1 and HPAFII) in GSE140077 and GSE106336 datasets (Figure 7B). The results revealed that Gemcitabine-resistant group had significantly higher metabolic risk scores than parental group (p-valve<0.001).

To investigate how the risk model leads to Gemcitabine resistance, we compared the expression of previous reported genes regulating Gemcitabine drug metabolism and efficacy (i.e. CDA, DCK, hENT1, hCNT1, RMM1, RMM2) between high-risk with low-risk group in TCGA cohort. The results showed that the mRNA levels of CDA (p-valve=0.001) and RMM2 (p-valve=2.517e-12) were significantly up-regulated in the high-risk group (Figure 7C).

Discussion

As the most frequently mutated oncogene in PC, KRAS and its downstream pathways affect several cellular processes including cell proliferation, migration, metabolism and autophagy in PC.[14] Small molecule drug (Sotorasib) that specifically and irreversibly inhibits KRAS had passed phase 1 clinical trial, showing a promising therapeutic prospect.[15] Studies have revealed oncogenic KRAS rewired metabolism to favor a more anabolic state and therefore promote tumorigenesis and progression of PC.[4] However, the molecular mechanisms were still not clear and it’s of great significance to conduct a profound study on KRAS-associated metabolic genes in PC.

In current study, we found out 6 differentially expressed metabolic genes based on KRAS mutation status, including CYP2S1, GPX3, FTCD, ENPP2, UGT1A10, and XDH. Then we developed a significant KRAS-associated metabolic risk model for prognostic prediction in PC. Further, the risk model was validated in two external cohorts (GSE57495 and GSE79668 datasets), which showed a stably high prognostic value for PC. These results indicate our model is accurate in predicting patients' survival, which may be helpful in designing personalized therapy targeting metabolism reprogramming. In the risk model, high expression of CYP2S1, GPX3 and FTCD were correlated with better prognoses, while high expressions of ENPP2, UGT1A10 and XDH were correlated with poor prognoses in PC. As a member of Cytochrome P450 (CYPs), CYP2S1 was known to biotransformate some exogenous and endogenous compounds including

drugs, fatty acids, and cholesterols.[16] Study showed that expressions of 8 CYPs were changed in CYP2S1-depleted cells and all of them were involved in lipid biotransformation. Indeed, CYP2S1 affected the metabolism of arachidonic acid (AA) and linoleic acid (LA) to modulate BEAS-2B cells growth.[17] In consistent with their results, our data also found CYP2S1 was enriched in Lipid metabolic process. It will be interesting to investigate the detailed mechanism by which CYP2S1 regulate lipid metabolism in PC in future study. As a member of glutathione peroxidase family (GPXs), GPX3 reduces glutathione to catalyze the reduction of hydrogen peroxide, hydroperoxides, and lipid hydroperoxides.[18] Low GPXs activity was found associated with enhanced lipid peroxidation in metastatic cancers, indicating that loss of GPX3 may promote systemic oxidative stress.[19] Studies had reported that low expression of GPX3 was associated with poor prognosis and chemoresistance in several tumor types.[18] Our results also proved that it's associated with a good outcome and may involve in "Lipid metabolic process and Glutamate receptor activity" in PC. ENPP2, which encodes an ecto-lysophospholipase D called Autotaxin (ATX), was found significantly increased in several types of cancer including PC.[20–22] Overwhelming evidences revealed that the ATX/lysophosphatidate (LPA) signaling axis serves key roles in energy metabolism regulation and obesity control, dysregulation of which could cause inflammation and tumorigenesis.[23, 24] Studies have indicated that ATX/LPA axis promoted DNA synthesis, proliferation, and invasion in pancreatic cancer cells via ERK1/2 and Rho pathways.[25, 26] Over-expression of ATX was also proven to led to elevated tumorigenesis and invasiveness compared with control groups in RAS-mutated NIH3T3 murine fibroblasts.[27] Further studies are needed to explore the relationship and underlying regulation mechanism of KRAS mutation and ENPP2/ATX in PC tumorigenesis and metabolism reprogramming.

UGT1A10 was an extrahepatic phase II metabolizing enzyme that expressed highly in numerous target areas for tobacco-induced cancers, including the upper aerodigestive tract.[28] UGT1A10 were elevated in a CPT-11/SN-38-resistant cell line and responsible for SN-38 glucuronidation, which was one of the mechanisms associated with irinotecan hydrochloride/7-ethyl-10-hydroaxycamptothecin (CPT-11/SN-38) resistance in lung cancer.[29] FTCD was found significantly down-regulated in hepatocellular carcinoma (HCC) and serve as a diagnostic biomarker for HCC.[30] Recently, FTCD was found to be associated with the sensitivity of chemotherapeutic drug methotrexate by using a CRISPR-Cas9-based screen.[31] XDH, a rate-limiting enzyme to catalyse the final steps of purine metabolism, was found significantly decreased and serves useful predictor of poor prognoses in several cancer types.[32] Uric acid, which was transformed by XDH, was found to modulate tumor cell sensitivity to the antimetabolite 5-FU, one of the most commonly used anticancer drugs in the clinic.[33] Our study consistently suggested that UGT1A10, FTCD, and XDH were involved in "Drug metabolism or Response to drug" in PC, but their specific roles in metabolic reprogramming and chemoresistance in PC still remain to be studied.

Gemcitabine based chemotherapy is a major treatment for PC patients with or without surgical resection. It has been reported Gemcitabine drug metabolism was affected by some genes or metabolites, which included drug transporters (i.e. hEN1, and hCNT1), activating and inactivating enzymes (i.e. CDA, and DCK) and competitive substrates to active metabolites (i.e. RRM1, and RRM2).[13] Cytidine deaminase (CDA) induced the deamination of dFdC to dFdU, leading to inactivation of gemcitabine.[34] It had been

confirmed that CDA expression was correlated with OS in PC and several in vitro studies revealed that over-expression of CDA led to gemcitabine resistance, while loss of CDA recovered gemcitabine sensitivity.[35, 36] RRM2 was involved in the activity of Ribonucleotide Reductase (RR), which was a rate-limiting enzyme of DNA synthesis. It has been demonstrated that up-regulation of RRM2 led to gemcitabine chemoresistance in PC cells and human PC xenografts in mice.[37] Besides, expression level of RRM2 was correlated inversely with OS in gemcitabine-treated PC patients in clinical study.[38] In current study, CDA and RRM2 were up-regulated in high-risk group evaluated by the risk model. Taken these together, the risk model may reflect the possibility of gemcitabine resistance, which would help oncologist choose appropriate chemotherapy regimen for PC.

There are a few limitations of our study. First, our study is mainly based on bioinformatic analysis and more experimental studies are needed to investigate how KRAS may regulate cancer cell metabolism in PC. Second, the established model needs to be further validated in prospective clinical studies.

In conclusion, we constructed and validated a prognostic model based on the KRAS-associated metabolic genes in PC. The prognostic model reflects tumor metabolic characteristics and Gemcitabine associated chemoresistance, which may have important value in aiding personalized therapy.

Abbreviations

PC-Pancreatic Cancer; OS - Overall Survival; AUC - Area Under the Curve; FPKM - Fragments Per Kilobase per Million; TCGA - the Cancer Genome Atlas; GEO - Gene Expression Omnibus; GSEA - Gene Set Enrichment Analysis; DEGs - Differentially Expressed Genes; PAGs - Prognosis-associated DEGs; ROC - Receiver Operating Characteristic; AJCC - American Joint Committee on Cancer; GO - Gene Ontology; KEGG - Kyoto Encyclopedia of Genes and Genomes.

Declarations

Ethics approval and consent to participate

This article did not contain any studies with human participants or animals performed by any of the authors. Therefore, local ethics approval was not needed.

Availability of data and materials

The data that support the findings of this study are available from TCGA (https://portal.gdc.cancer.gov/repository) , GEO (GEO, https://www.ncbi.nlm.nih.gov/geo/) and GSEA databases (https://www.gsea-msigdb.org/gsea/index.jsp).

Conflicts of interest

The authors declare that they have no potential conflicts of interest.

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Authors' contributions

Conceptualization, Zuyi Ma, Shanzhou Huang, Chuanzhao Zhang and Baohua Hou; Data curation, Zuyi Ma, Zixuan Zhou, Hongkai Zhuang; Formal analysis, Zuyi Ma, Zhenchong Li; Methodology, Zuyi Ma; Software, Zhenchong Li, Bowen Huang, Yuanfeng Gong, Chunsheng Liu, Yiping Zou, Zehao Zheng and LinLing Yang; Validation, Zuyi Ma, Zixuan Zhou and Shanzhou Huang; Visualization, Zuyi Ma; Writing-review, Zuyi Ma, Shanzhou Huang, Chuanzhao Zhang and Baohua Hou.

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Figures

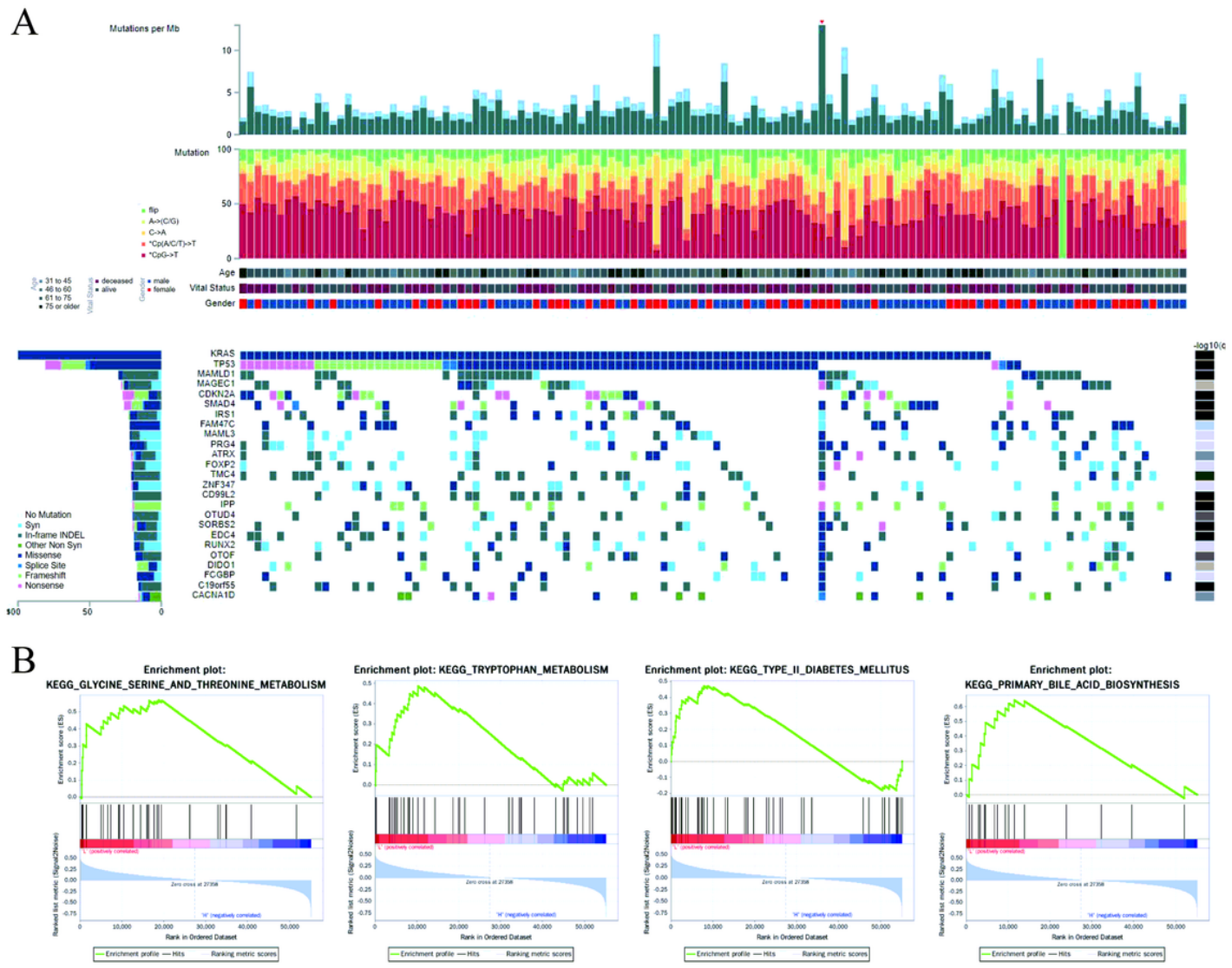

Figure 1

Gene set enrichment analysis (GSEA) of KRAS in TCGA Pancreatic Cancer (PC) cohort. (A) Genomic landscape and the mutational signatures of PC in the TCGA cohort (FireBrowse platform). (B) Significant enrichment of the metabolic-related phenotype in KRAS WT PC patients compared with KRAS MUT PC patients.

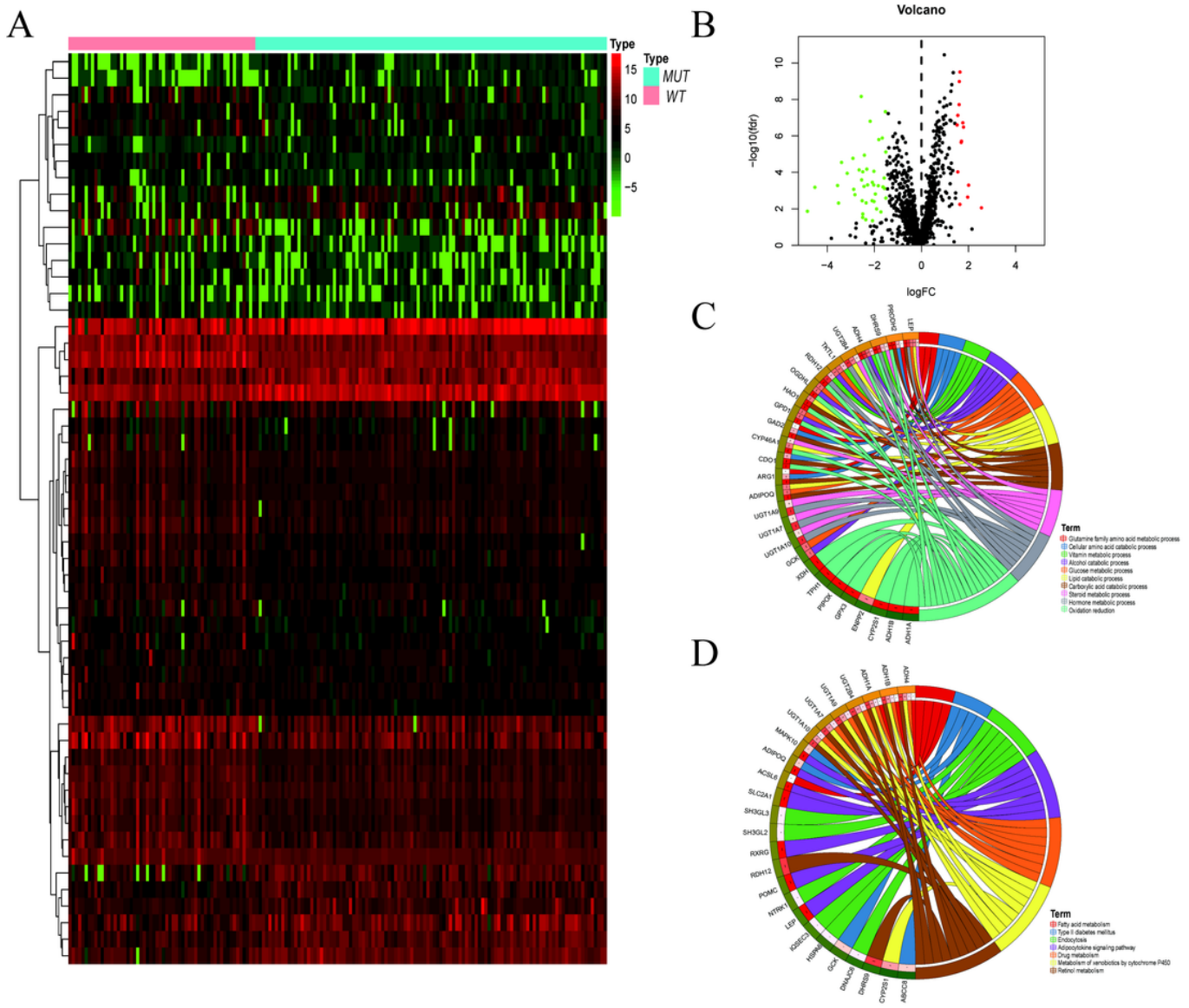

Figure 2

Identification and enrichment analysis of differentially expressed metabolic genes (DEGs) based on KRAS mutation status. (A-B) Heatmap (A) and Volcano plot (B) of 54 DEGs. (C) Gene Ontology (GO) enrichment analysis of DEGs. (D) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of DEGs.

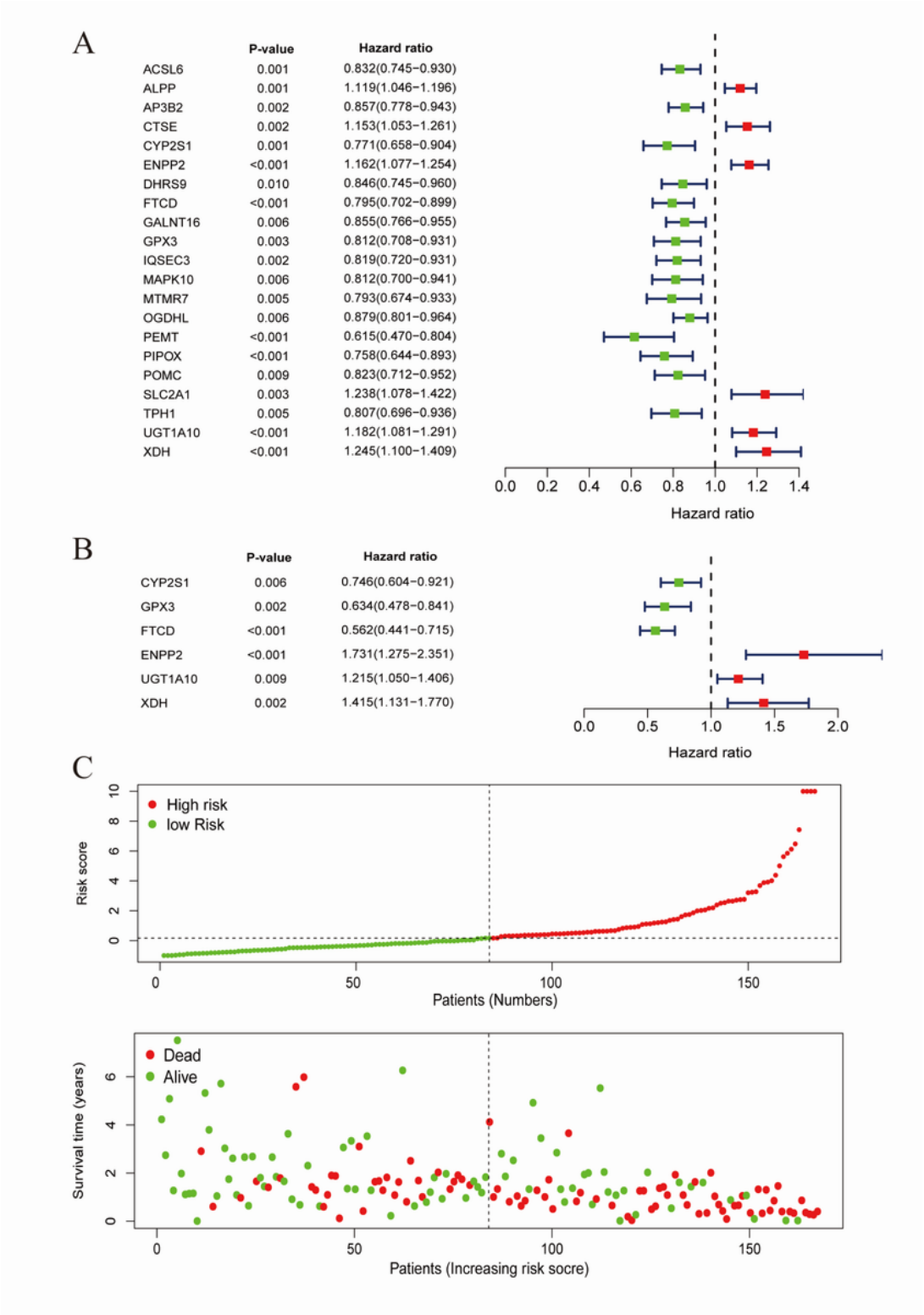

Figure 3

Construction of the KRAS-associated metabolic risk model for pancreatic cancer (PC). (A-B) Univariable (A) and multivariable (B) Cox regression analysis to select prognosis-associated DEGs (PAGs). (C) Distribution of risk score and patient survival time, and status of PC.

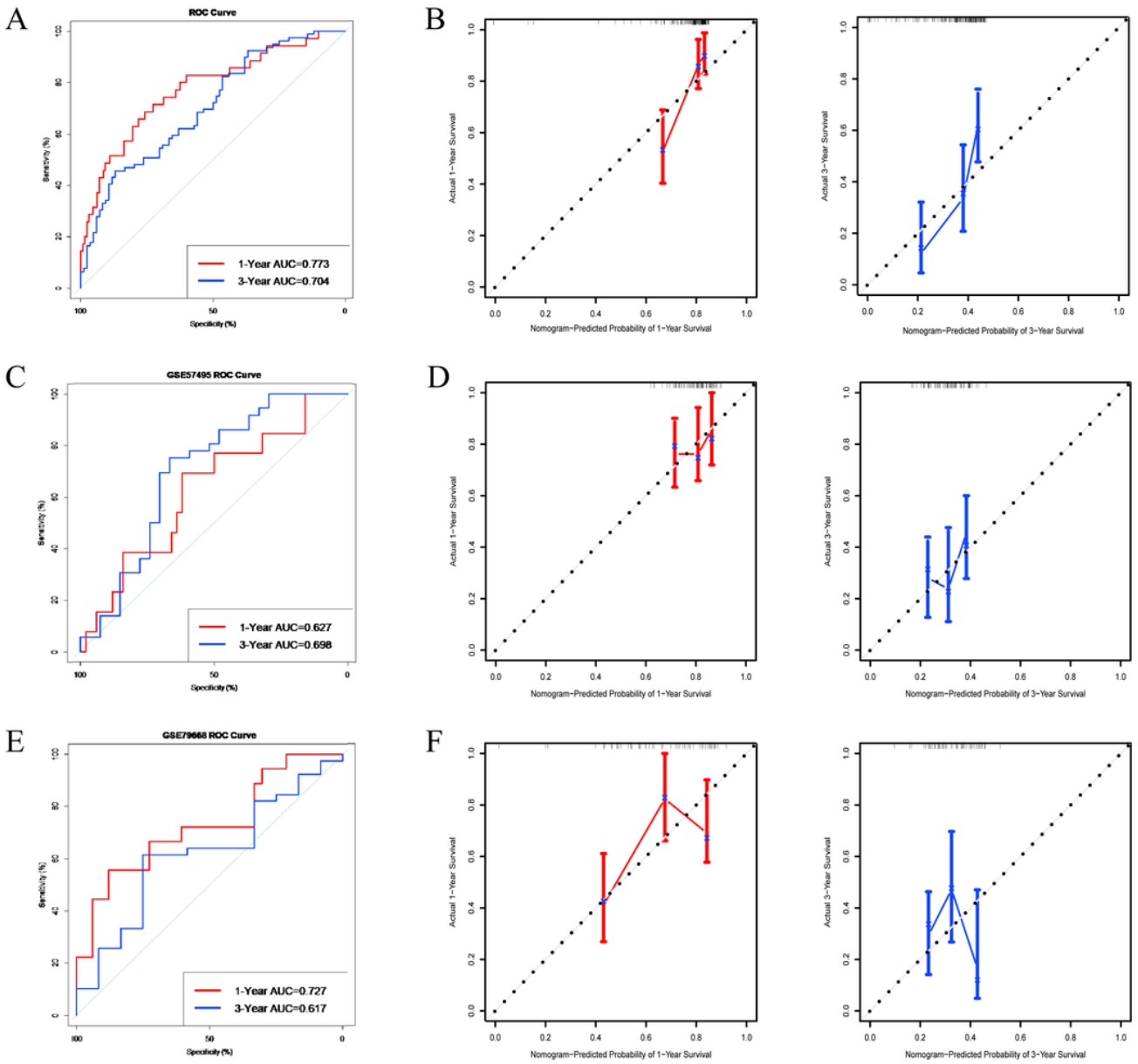

Figure 4

Prognostic analysis of the risk model. (A) Receiver operating characteristic (ROC) of the risk model for OS in TCGA cohort. Area under the curve (AUC) at the 1-, and 3-year survival times was 0.773 and 0.704. (B) Calibration curves of the risk model for 1-, and 3- year survival in TCGA cohort. (C) ROC of the risk model for OS in GSE57495 dataset. AUC at the 1-, and 3-year survival times was 0.627 and 0.698. (D) Calibration curves of the risk model for 1-, and 3- year survival in GSE79668 dataset. (E) ROC of the risk model for OS in GSE57495 dataset. AUC at the 1-, and 3-year survival times was 0.727 and 0.617. (F) Calibration curves of the risk model for 1-, and 3- year survival in GSE79668 dataset.

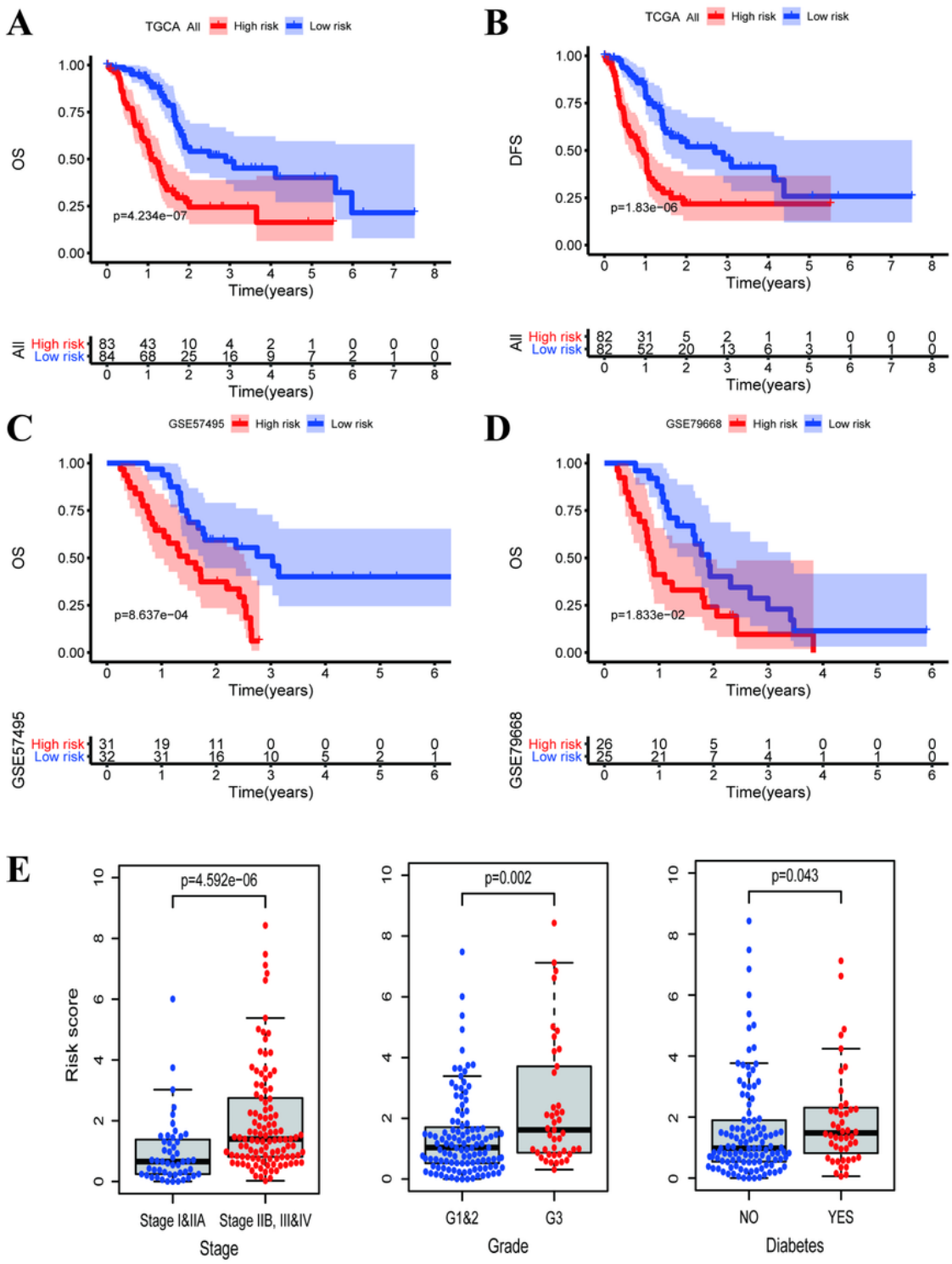

Figure 5

Association between the risk model with patients survival and clinicopathological characteristics in PC. (A-B) Kaplan-Meier (KM) analysis of TCGA pancreatic cancer patients was stratifed by median risk. (A) Overall survival (OS) was significantly higher in the low-risk group than in the high-risk group. (B) Disease-free survival (DFS) was significantly higher in the low-risk group than in the high-risk group. (C) KM analysis of OS in GSE57495 dataset. (D) KM analysis of OS in GSE79668 dataset. (E) Patients with

advanced stage (p-valve=4.592e-06), higher histologic grade (p-valve=0.002) or diabetes history (p-valve=0.043) had higher risk scores.

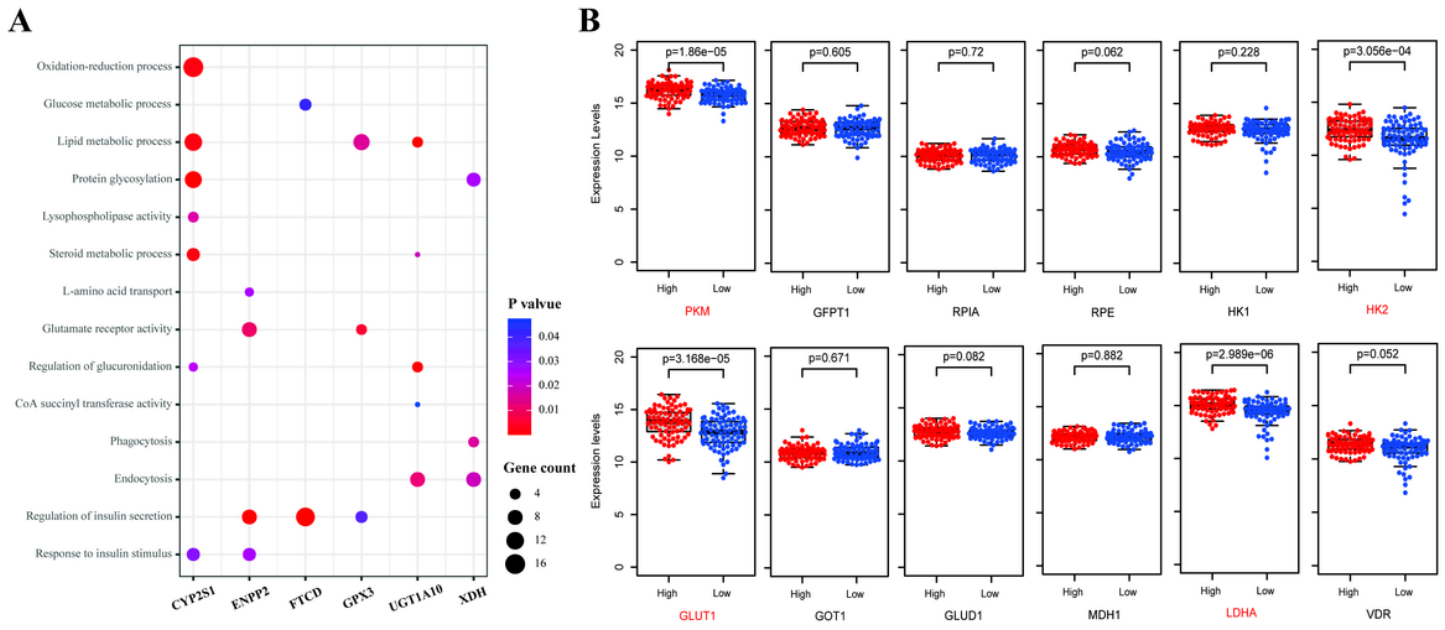

Figure 6

Association between the risk model with metabolic characteristics of PC. (A) Enrichment analysis of 6 PAGs showed the potential metabolic pathways involved. (B) The expression levels of several KRAS-driven metabolic genes between high-risk with low-risk group in TCGA cohort. Higher expressions of PKM (p=1.86e-05), GLUT1 (p=3.168e-05), HK2 (p=3.056e-04), LDHA (p=2.989e-06), and VDR (p=0.012) were found in the high-risk group.

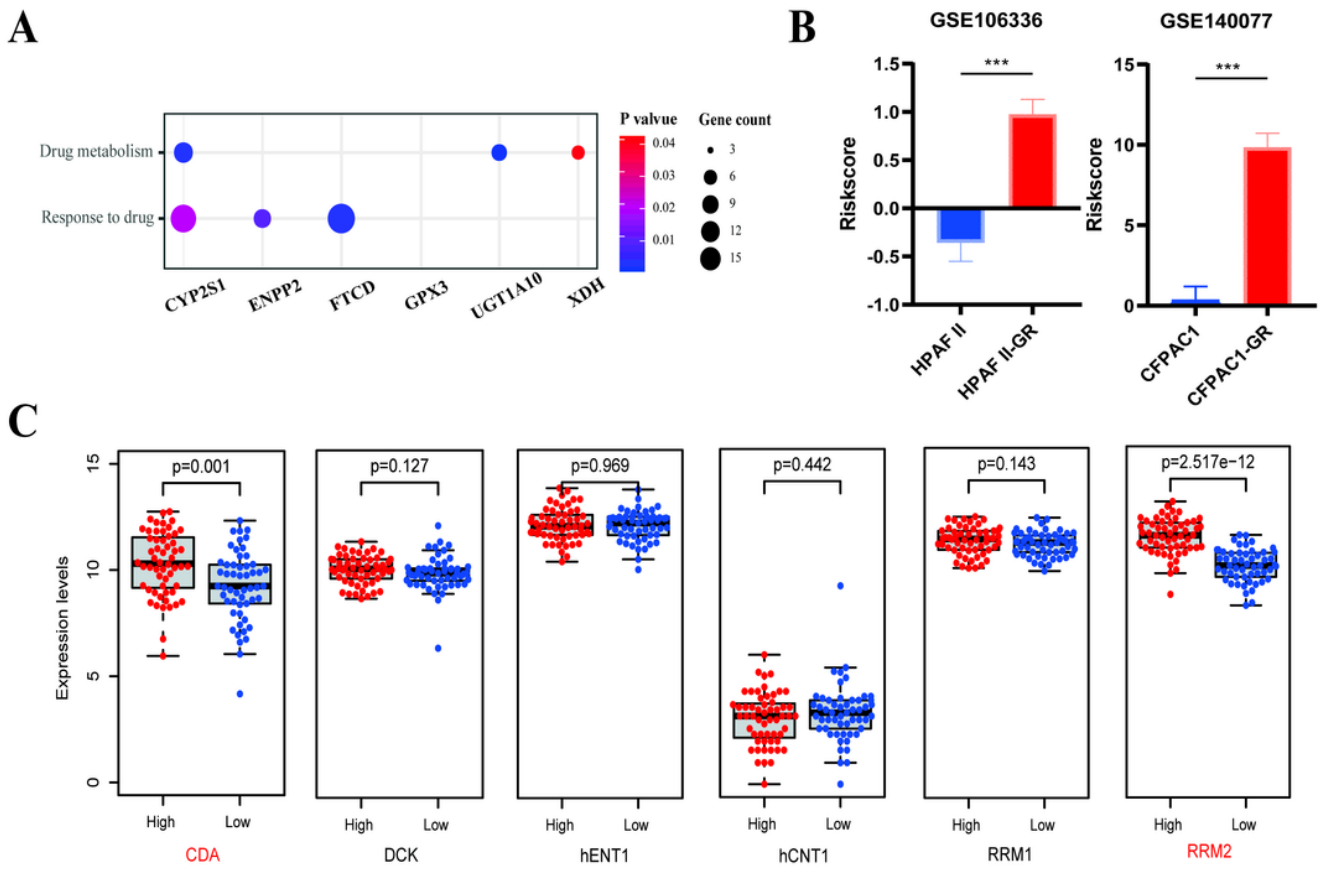

Figure 7

Association between the risk model with Gemcitabine chemoresistance in PC. (A) Enrichment analysis showed 5 of 6 PAGs involved in “Drug metabolism or Response to drug”. (B) Gemcitabine-resistant pancreatic cancer cell lines (CFPAC-1 and HPAFII) had higher risk scores than parental group (p-valve<0.001). (C) The expression levels of several Gemcitabine metabolism-associated chemoresistance genes between high-risk with low-risk group in TCGA cohort. Higher expressions of CDA (p-valve=0.001) and RMM2 (p-valve=2.517e-12) were found in the high-risk group.