

Identification of key pathways and candidate genes in pancreatic ductal adenocarcinoma using bioinformatics analysis

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Abstract. Pancreatic ductal adenocarcinoma (PDAC) is a malignant tumor with a high degree of malignancy that is difficult to diagnose and treat. The present study integrated PDAC cohort profile datasets to identify key candidate genes and pathways involved in the pathogenesis of the disease. The expression profiles of GSE28735 included 45 PDCA and matching pairs of adjacent non-tumor tissue. Differentially expressed genes (DEGs) were sorted and candidate genes and pathway enrichment were analyzed. A DEG-associated protein-protein interaction (PPI) network was constructed. A total of 424 DEGs were identified in PDAC, including 159 upregulated genes and 265 downregulated genes. Gene Ontology analysis results indicated that upregulated DEGs were significantly enriched in biological process, molecular function and cellular component categories. Kyoto Encyclopedia of Genes and Genomes pathway analysis demonstrated that the upregulated DEGs were enriched in 'pancreatic secretion', 'protein digestion' and 'absorption'. Downregulated DEGs were enriched in 'ECM-receptor interaction', 'focal adhesion' and 'PI3K/AKT' signaling pathways. The PPI network revealed that these genes were involved in significant pathways, including 'ECM organization' signaling pathways (Hippo signaling pathway, TGF- β signaling pathway, Hedgehog signaling pathway and Wnt signaling pathway), 'serine-type peptidase activity' signaling pathway (PI3K-Akt signaling pathway, TNF- α signaling pathway and Wnt signaling pathway) and 'extracellular region' signaling

pathways (RTP signaling pathway, G protein-coupled receptor signaling pathway and RAS-RAF-MAPK signaling pathway). The identification of these candidate genes and pathways sheds light on the etiology and molecular mechanisms of PDAC and may guide the development of novel therapies for pancreatic cancer.

Introduction

Pancreatic cancer has a poor prognosis, with a median survival time of 3-6 month and a 5-year survival rate of less than 5% (1-3). The most common type of pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC), accounting for ~90% of pancreatic cancer cases (4). Although numerous studies have focused on the pathogenesis and progression of pancreatic cancer, the etiology and molecular mechanisms of pancreatic cancer remain unclear (5,6). Previous scientific studies have demonstrated that the occurrence and progression of pancreatic cancer involve the interaction of several factors, including gene mutations and environmental conditions (7,8). Thus far, there remains a lack of information regarding the molecular mechanisms that cause the development and progression of pancreatic cancer that would allow for improved precision therapies. Therefore, understanding the molecular mechanisms of pancreatic cancer can provide an effective basis for early prevention, diagnosis and treatment.

The advent of the gene chip and high-throughput gene analysis platforms allows for the rapid detection of gene expression in a microarray, which is particularly suitable for screening differentially expressed genes (DEGs) (9). With the widespread application of gene chip technology in cancer research, a large amount of genetic data has been produced and stored in public gene databases. Classification, integration and analysis of these data can provide valuable insights and evidence for cancer research. In the past few years, numerous gene chip expression profiles have been used to study the pathogenesis and development of PDAC and hundreds of DEGs have been identified (10). However, due to differences in sample size and limitations of the studies, no reliable biomarkers were identified. The combination of gene chip and biological information analysis technology can be used to monitor the expression of DEGs in the development and

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progression of PDAC and to elucidate the signaling pathways involved, potentially revealing targets which can be modulated to treat PDAC (11).

In the present study, the original GSE28735 data set (12) was downloaded from the Gene Expression Omnibus (GEO) database (13). The dataset contained the gene expression profiles of 45 matching pairs of pancreatic tumor and adjacent non-tumor tissues from 45 patients with PDAC. DEGs were detected by comparing the gene expression profiles between tumor tissues and paracancerous tissues in patients with PDAC. Subsequently, the DEGs were filtered using the Morpheus website (<https://software.broadinstitute.org/morpheus/>) with data processing standard. Then, the DEGs were screened using the Gene-Spring software (version 11.5; Agilent Technologies, Inc., Santa Clara, CA, USA), followed by Gene Ontology (GO); (www.geneontology.org) and pathway enrichment analysis. In addition, a protein-protein interaction (PPI) network was established and three significant modules were analyzed. The analysis of the biological pathways underlying the development of PDAC may provide information for its diagnosis, prognosis and treatment.

Materials and methods

Microarray data. The gene expression profiles of the GSE28735 dataset were downloaded from the GEO database. The GPL6244 [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array platform (Affymetrix; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used. The GSE28735 dataset contained 90 samples, including 45 PDAC tumor samples and 45 matching pairs of adjacent non-tumor tissue samples.

Identification of DEGs in GSE28735. The raw expression data files include TXT files (Affymetrix platform) used for analysis by processing using the Morpheus website. Data were categorized into two groups with similar expression patterns in PDAC tumor samples and matching pairs of adjacent non-tumor tissue samples. A t-test was used to identify the DEGs and \log_2 fold change ≥ 1 and $P < 0.05$ were considered statistically significant.

Gene ontology and pathway enrichment analysis of DEGs. GO analysis was used to annotate genes and classify up and downregulated DEGs. GO terms are divided into three main categories: Biological process (BP), cellular component (CC) and molecular function (MF). The Kyoto Encyclopedia of Genes and Genomes (KEGG; www.kegg.jp) website is an online database which contains defined and associated gene sets and their pathways. The Database for Annotation, Visualization and Integrated Discovery (DAVID; david.ncifcrf.gov) allows analysis of gene lists and provides biological information regarding genes. To analyze the upregulated and downregulated genes in DEGs, GO and KEGG pathway analysis were used in the DAVID database. $P < 0.05$ was considered to indicate a statistically significant difference.

Integration of PPI network. The Search Tool for the Retrieval of Interacting Genes (STRING; www.string-db.org) was used to evaluate the PPI information. The PPI

network served to identify the key genes and Cytoscape software (version 3.5.1; www.cytoscape.org) was used to draw the network diagram. The topology of the PPI network was analyzed and the extent of the expression of each gene was calculated. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Identification of DEGs in pancreatic cancer. A total of 45 PDAC tumor samples and 45 matching pairs of adjacent non-tumor tissue samples were analyzed. A total of 424 DEGs were identified from GSE28735, including 159 upregulated and 265 downregulated genes (Table I). The heat map of DEG expression, presenting the top 50 upregulated and 50 downregulated genes was constructed using the web-based tool Morpheus (Fig. 1).

GO term and pathway enrichment analyses. To further elucidate the function of the selected genes, the online software DAVID was used to perform DEG GO analysis. As aforementioned, GO analysis results classify DEG functions and pathways into three functional groups: BP, CC and MF. For BP, the upregulated DEGs were enriched in 'digestion', 'lipid digestion' and 'proteolysis', while the downregulated DEGs were enriched in 'ECM organization', 'extracellular structure organization' and 'cell adhesion' (Tables II and III). For CC, the upregulated DEGs were enriched in the 'extracellular region', and the downregulated DEGs were enriched in 'extracellular region' and 'ECM' (Tables II and III). For MF, the upregulated DEGs were enriched in 'serine-type peptidase activity', 'serine hydrolase activity' and 'peptidase activity', and the downregulated DEGs were enriched in 'ECM structural constituent', 'integrin binding' and 'cell adhesion molecule binding' (Tables II and III).

KEGG pathway analysis in pancreatic cancer. KEGG pathway analysis was used to analyze the most significantly enriched pathways of the upregulated DEGs and downregulated DEGs. The upregulated DEGs were enriched in 'pancreatic secretion', 'protein digestion and absorption' and 'fat digestion and absorption' (Table IV). The downregulated DEGs were enriched in 'ECM-receptor interaction', 'focal adhesion' and 'PI3K/Akt signaling' pathways (Table IV).

PPI and modular analysis in pancreatic cancer. Using the STRING online database and Cytoscape software analysis, a total of 386 DEGs (143 upregulated and 243 downregulated genes) of the 424 commonly altered DEGs were filtered into the DEGs PPI network complex, including 424 nodes and 1090 edges (Fig. 2A). The 10 nodes with the highest degree were cystic fibrosis transmembrane conductance regulator (CFTR), SLC7A2 (solute carrier family 7 member 2), C-C motif chemokine ligand 18 (CCL18), pyruvate dehydrogenase kinase 4 (PDK4), BAI1 associated protein 2 like 1 (BAIAP2L1), integrin subunit $\alpha 3$ (ITGA3), carboxypeptidase A1 (CPA1), G protein-coupled receptor class C group 5 member A (GPC5A), serine/threonine/tyrosine kinase 1 (STYK1), and ST6 N-acetylgalactosaminide α -2, 6-sialyltransferase 1 (ST6GALNAC1). Among the upregulated DEGs, a total of

Table I. A total of 424 DEGs were identified from the GSE28735 dataset, including 159 upregulated genes and 265 downregulated genes in PDAC tissues, compared to adjacent non-tumor tissue samples.

Differential expression	Gene symbol
Upregulated	EPB41L4B, FAM129A, SLC1A2, KLKB1, ALDH1A1, PAH, CHGA, CHST9, SEMA6A, SERPINA5, KIF1A, CHRDL1, SLC16A10, CLU, MIR27B, PRKAR2B, FAM3B, ADHFE1, LONRF2, DPT, CHRM3, SLC3A1, ABAT, PPY, BNIP3, NUCB2, GPHA2, ATRNL1, ESRRG, ABCA8, FAM150B, ONECUT1, PRSS3P2, OR4D5, CXCL12, IL22RA1, TSPAN7, F8, GCG, ADGRV1, SV2B, UGT2B11, SPINK1, PROX1, ANGPTL1, UNC79, AMY2B, MCOLN3, AQP12B, FAM159B, FOSB, BTG2, SLC43A1, FLRT2, GSTA1, AQP12B, C5, SCG3, CCDC141, DPP10, PKHD1, PRSS3, C2CD4B, MT1G, HOMER2, GRB14, LYVE1, BACE1, SLC39A5, CD36, RGN, SYCN, GC, EPHX2, REG3G, DCDC2, GUCA1C, SST, PCSK1, PDZK1P1, BEX1, PRSS2, LIFR, GRPR, SLC30A8, MIR217, LMO3, ANKRD62, CTNND2, PM20D1, CFTR, GNMT, TFPI2, SLC17A4, PAK3, GSTA2, AMY2B, G6PC2, TTN, CELP, SLC4A4, PRSS3P2, C6, TTR, QP8, SLC7A2, KCNJ16, PDK4, OR8D4, REG3A, FABP4, NRCAM, NRG4, PAIP2B, GATM, FGL1, ACADL, ADH1B, TRHDE, RBPJL, SCGN, REG1A, PRSS1, CPB1, SLC16A12, ANPEP, TMED6, KLK1, RO1B, F11, CTRB2, AOX1, NR5A2, KIAA1324, CELA3B, EGF, CPA1, PDIA2, REG1CP, EG1B, PNLIP, CTRB1, CTRL, CELA3A, CELA2B, CELA2A, PLA2G1B, SERPINI2, CLPS, ERP27, FAM24B, ALB, CPA2, CEL, GP2, CTRC, IAPP, PNLIPRP2, PNLIPRP1
Downregulated	CEACAM5, SLC6A14, LAMC2, GALNT5, TSPAN1, CTSE, POSTN, CEACAM6, ANXA10, LAMB3, ITGA2, TMPRSS4, FN1, COL11A1, SERPINB5, DPCR1, AGR2, CLDN18, ITGB6, KRT19, GABRP, CST1, VSIG1, SULF1, TFF1, COL17A1, SLC2A1, PLAC8, CEMIP, SLPI, CP, AHNAK2, MMP12, COL12A1, TMC5, VCAN, MUC17, KRT7, ANLN, INHBA, TRIM31, LIPH, CDH3, TRIM31, SCEL, NOX4, THBS2, EGLN3, C13, ADGRF1, MBOAT2, ANTXR1, TCN1, ANKRD22, COL10A1, CXCL5, XYD3, KRT17, BCAS1, ITGA3, SDR16C5, EDIL3, APOL1, UGT1A3, COL1A1, MMP11, FERMT1, FAP, ANXA8L1, CDH11, COL1A2, MET, FNDC1, FBXO32, COMP, NQO1, ACSL5, MLPH, NPR3, ANXA8L1, MIA-RAB4B, COL8A1, GCNT3, IGFL2, ADAMTS12, TNS4, CAPG, TRIM29, TSPAN8, CYP2C18, TRIM31, TMEM45B, MATN3, COL5A2, PLAUI, PADI1, ITGA11, COL3A1, CCL20, IGFP5, LAMA3, HK2, IFI27, MYOF, PLAT, FER1L6, KRT6C, ECT2, LY75, MMP14, TOP2A, DNRA, LEF1, CENPF, TNFAIP6, ITGB4, PLEK2, CEACAM1, LAMP5, TMC7, NPR3, OLR1, SERPINB3, ANO1, DHRS9, SLC6A6, MICAL2, MUC16, ARNTL2, PTPRR, KYNU, NRP2, S100A14, CD109, BAIAP2L1, AFAP1-AS1, LOXL2, FGD6, CST2, IFI44L, S100P, MMP1, COL6A3, SL44A4, ERO1A, ASPM, BGN, DKK1, STYK1, MMP7, RUNX2, NT5E, TGM2, HEPH, KRT17, GPX2, OSBPL3, LMO7, GPRC5A, EPHA4, DCP1, GF2BP3, S100A16, PXDN, MKI67, EFNA5, KRT17, MELK, ADAM9, SLC22A3, MST1R, ACTA2, FF2, LCN2, PLPP4, ADAM28, MXRA5, DPYSL3, TGFBI, XDH, CCL18, OAS1, ABHD17C, RHBDL2, HIST1H3H, MUC1, INPP4B, AEBP1, MMP9, MTMR11, FOXQ1, ENO2, OCIAD2, DLGAP5, HPGD, TPX2, PLA2R1, SRPX2, LRRN1, SLC01B3, SEMA3C, IL1RAP, SYTL2, FER1L4, DSG2, SULF2, HOXB5, MFP5, IL2RG, SULT1B1, CORIN, SLC9A2, GJB2, ADAM12, PLS1, AK4, ATP2C2, GREM1, ETV1, LTBP1, OAS2, ASAP2, SGIP1, PGM2L1, DDX60, DGKH, KCNN4, MALL, P4HA1, ANXA3, TSK, EPYC, NRP2, FUT3, ADAMTS6, KRT6A, IL1R2, DCBLD2, NMU, EFNB2, ST6GALNAC1, ANGPT2, FCGR3B, KIF23, FBN1, PKM, SEMA7A, TRIM16, RTKN2, SLC26A9, NTM, PCDH7, RAI14, SULT1C2, ESM1, AREG, DSG3, GPX8, MACC1, CTHRC1, HIST1H3I, SCNN1A, SLC16A3

DEGs, differentially expressed genes; PDAC, pancreatic ductal adenocarcinoma.

143 DEGs were filtered into the DEG PPI network complex including 143 nodes and 263 edges (Fig. 2B), which were mainly associated with ‘digestion’, ‘serine-type peptidase

activity’ and the ‘extracellular region’ (Table V). Among the downregulated DEGs, a total of 143 DEGs were filtered into the DEGs PPI network complex including 243 nodes and

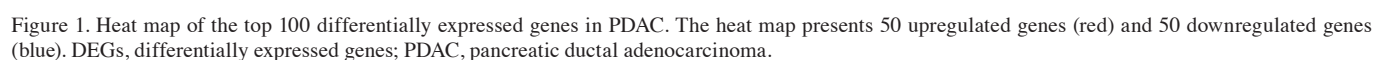


Table II. GO analysis of upregulated DEGs associated with PDAC.

Category	Term	Gene function	Count	P-value
BP	GO:0007586	Digestion	18	3.31×10^{-14}
BP	GO:0044241	Lipid digestion	6	9.18×10^{-7}
BP	GO:0006508	Proteolysis	35	1.08×10^{-6}
BP	GO:0006766	Vitamin metabolic process	9	1.57×10^{-5}
BP	GO:0009235	Cobalamin metabolic process	5	2.56×10^{-5}
BP	GO:0015850	Organic hydroxy compound transport	10	4.03×10^{-5}
BP	GO:0006767	Water-soluble vitamin metabolic process	7	9.77×10^{-5}
BP	GO:0006629	Lipid metabolic process	26	1.11×10^{-4}
BP	GO:0046903	Secretion	23	1.23×10^{-4}
BP	GO:0032940	Secretion by cell	21	1.67×10^{-4}
CC	GO:0005576	Extracellular region	90	1.47×10^{-17}
CC	GO:0005615	Extracellular space	47	4.64×10^{-15}
CC	GO:0044421	Extracellular region part	78	8.75×10^{-15}
CC	GO:0031988	Membrane-bounded vesicle	64	6.61×10^{-9}
CC	GO:0070062	Extracellular exosome	52	1.46×10^{-7}
CC	GO:1903561	Extracellular vesicle	52	1.72×10^{-7}
CC	GO:0043230	Extracellular organelle	52	1.74×10^{-7}
CC	GO:0030141	Secretory granule	15	4.27×10^{-6}
CC	GO:0060205	Cytoplasmic membrane-bounded vesicle lumen	8	4.51×10^{-5}
CC	GO:0031983	Vesicle lumen	8	4.80×10^{-5}
MF	GO:0008236	Serine-type peptidase activity	18	2.13×10^{-10}
MF	GO:0017171	Serine hydrolase activity	18	2.51×10^{-10}
MF	GO:0008233	Peptidase activity	27	3.41×10^{-10}
MF	GO:0004252	Serine-type endopeptidase activity	17	4.20×10^{-10}
MF	GO:0070011	Peptidase activity, acting on L-amino acid peptides	26	8.65×10^{-10}
MF	GO:0004175	Endopeptidase activity	18	8.45×10^{-7}
MF	GO:0008238	Exopeptidase activity	9	4.27×10^{-6}
MF	GO:0008235	Metalloexopeptidase activity	6	1.98×10^{-4}
MF	GO:0004806	Triglyceride lipase activity	4	7.93×10^{-4}
MF	GO:0005179	Hormone activity	6	3.54×10^{-3}

GO, gene ontology; DEGs, differentially expressed genes; PDAC, pancreatic ductal adenocarcinoma; BP, biological process; MF, molecular function; CC, cellular component.

497 edges (Fig. 2C), which were mainly associated with 'ECM organization', 'ECM structural constituents' and the 'extracellular region' (Table VI).

Discussion

The incidence of PDAC is increasing worldwide (14). The clinical signs and symptoms may be difficult to diagnose in the initial stages of the disease (7). Patients are often diagnosed at a late stage, when regional invasion or distant metastasis have occurred, resulting in a 5-year survival rate of ~5% (15,16). An insight into the molecular mechanisms of PDAC would allow for earlier diagnosis and more effective treatment. The rapid development of gene chips and high-throughput sequencing can rapidly and accurately provide gene expression data for

thousands of genes in the human genome. Previous studies have identified some of the genes and signaling pathways that serve a role in the development of pancreatic cancer from chip analysis (17,18). In the present study, the chip data in the GSE28735 dataset was comprehensively analyzed and 424 common DEGs (159 upregulated and 265 downregulated) between PDAC and matching pairs of adjacent non-tumor tissue were identified using bioinformatics analysis.

GO analysis is an international standardized gene function classification system that provides the molecular function of genes involved in a variety of biological processes (19). In the current study, GO term analysis revealed that the upregulated genes were mainly involved in 'digestion', 'lipid digestion' and 'proteolysis', and downregulated DEGs were involved in 'extracellular matrix organization', 'extracellular structure

Table III. GO analysis of downregulated DEGs associated with PDAC.

Category	Term	Gene function	Count	P-value
BP	GO:0030198	Extracellular matrix organization	42	2.61x10 ⁻²⁷
BP	GO:0043062	Extracellular structure organization	42	2.94x10 ⁻²⁷
BP	GO:0007155	Cell adhesion	67	2.12x10 ⁻¹⁵
BP	GO:0022610	Biological adhesion	67	2.52x10 ⁻¹⁵
BP	GO:0016477	Cell migration	53	3.81x10 ⁻¹⁴
BP	GO:0030574	Collagen catabolic process	15	1.91x10 ⁻¹³
BP	GO:0051674	Localization of cell	55	2.69x10 ⁻¹³
BP	GO:0048870	Cell motility	55	2.69x10 ⁻¹³
BP	GO:0044243	Multicellular organism catabolic process	15	8.59x10 ⁻¹³
BP	GO:0006928	Movement of cell or subcellular component	64	1.18x10 ⁻¹²
CC	GO:0005576	Extracellular region	148	4.33x10 ⁻²⁵
CC	GO:0044421	Extracellular region part	133	3.86x10 ⁻²⁴
CC	GO:0031012	Extracellular matrix	42	8.29x10 ⁻¹⁸
CC	GO:0005578	Proteinaceous extracellular matrix	35	1.90x10 ⁻¹⁷
CC	GO:0044420	Extracellular matrix component	19	1.21x10 ⁻¹²
CC	GO:0005615	Extracellular space	59	6.91x10 ⁻¹²
CC	GO:0070062	Extracellular exosome	84	6.11x10 ⁻¹⁰
CC	GO:1903561	Extracellular vesicle	84	7.84x10 ⁻¹⁰
CC	GO:0043230	Extracellular organelle	84	7.99x10 ⁻¹⁰
CC	GO:0031988	Membrane-bounded vesicle	96	9.00x10 ⁻⁹
MF	GO:0005201	Extracellular matrix structural constituent	13	1.27x10 ⁻⁹
MF	GO:0005178	Integrin binding	14	2.41x10 ⁻⁹
MF	GO:0050839	Cell adhesion molecule binding	25	2.52x10 ⁻⁸
MF	GO:0019838	Growth factor binding	13	2.08x10 ⁻⁷
MF	GO:0004222	Metalloendopeptidase activity	12	4.62x10 ⁻⁷
MF	GO:0005539	Glycosaminoglycan binding	15	1.20x10 ⁻⁶
MF	GO:0005509	Calcium ion binding	28	2.19x10 ⁻⁶
MF	GO:0005518	Collagen binding	9	2.67x10 ⁻⁶
MF	GO:0005102	Receptor binding	43	3.59x10 ⁻⁶
MF	GO:0008237	Metallopeptidase activity	13	1.34x10 ⁻⁵

GO, gene ontology; DEGs, differentially expressed genes; PDAC, pancreatic ductal adenocarcinoma; BP, biological process; MF, molecular function; CC, cellular component.

organization' and 'cell adhesion'. The pancreas mainly secretes trypsin and pancreatic lipase and abnormalities in secretions can interfere with protein and lipid metabolism, leading to chronic pancreatitis which is one of the important contributing factors for pancreatic cancer (20). The stability of cell structure and cell adhesion is also a major factor in the formation of pancreatic cancer (21).

Furthermore, KEGG pathway analysis indicated that the upregulated DEGs were involved in pancreatic secretion pathways and protein and lipid digestion and absorption pathways. Existing studies revealed that metabolic change is considered one of the characteristics of cancer, especially the dysfunction of pancreatic secretion (11,22). In pancreatic cancer, metabolic changes are prominent in protein and lipid digestion and absorption pathways (23). The downregulated DEGs were

associated with 'ECM-receptor interaction', 'focal adhesion' and the 'PI3K-Akt signaling' pathways.

Previous studies indicated that pancreatic stellate cells, which can cause pancreatic fibrosis leading to pancreatic cancer, can produce and secrete ECM (24,25). One of the components of ECM, hyaluronic acid, can combine with CD44 antigen and influence vascular epithelial-mesenchymal transition (EMT) as well as cancer cell resistance to chemotherapy (4). Furthermore, the main constitutive protein of ECM, collagen I, can promote the adhesion of pancreatic cancer cells through the proliferation and migration of integrin $\alpha 2\beta 1$ (24). Collagen, fibronectin and laminin are also associated with chemoresistance in pancreatic cancer cells *in vitro* (26). Previous studies revealed that focal adhesions interact with the ECM and can promote EMT,

Table IV. KEGG pathway analysis of DEGs associated with PDAC.

A, Upregulated					
Pathway	Name	Count	P-value	Genes	
hsa04972	Pancreatic secretion	19	4.1x10 ⁻¹⁸	PNLIP, CELA3A, PNLIPRP1, CELA3B, PNLIPRP2, PRSS1, CFTR, CEL, CHRM3, PRSS2, PRSS3, CPA2, PLA2G1B, CELA2B, CELA2A, CPA1, CPB1, SLC4A4, CTRL	
hsa04974	Protein digestion and absorption	13	1.9x10 ⁻¹⁰	CELA3A, CELA3B, SLC16A10, PRSS2, PRSS3, PRSS1, CPA2, CELA2B, CELA2A, CPA1, SLC3A1, CPB1, CTRL	
hsa04975	Fat digestion and absorption	7	4.1x10 ⁻⁶	PNLIP, CEL, CLPS, PNLIPRP1, PNLIPRP2, CD36, PLA2G1B	
hsa04610	Complement and coagulation cascades	6	1.0x10 ⁻³	F11, KLKB1, SERPINA5, C6, C5, F8	
hsa00982	Drug metabolism-cytochrome P450	5	7.1x10 ⁻³	GSTA1, GSTA2, AOX1, UGT2B11, ADH1B	
hsa00561	Glycerolipid metabolism	4	2.7x10 ⁻²	PNLIP, CEL, PNLIPRP1, PNLIPRP2	
hsa04950	Maturity onset diabetes of the young	3	3.4x10 ⁻²	ONECUT1, IAPP, NR5A2	
hsa00830	Retinol metabolism	4	3.7x10 ⁻²	ALDH1A1, AOX1, UGT2B11, ADH1B	
hsa04971	Gastric acid secretion	4	4.9x10 ⁻²	KCNJ16, CHRM3, CFTR, SST	
hsa00980	Metabolism of xenobiotics by cytochrome P450	4	5.1x10 ⁻²	GSTA1, GSTA2, UGT2B11, ADH1B	
B, Downregulated					
Pathway	Name	Count	P-value	Genes	
hsa04512	ECM-receptor interaction	17	1.4x10 ⁻¹³	COL3A1, ITGB4, ITGA11, ITGA2, ITGA3, COL5A2, LAMB3, LAMA3, COMP, ITGB6, COL6A3, COL1A2, LAMC2, COL1A1, THBS2, COL11A1, FN1	
hsa04510	Focal adhesion	18	1.1x10 ⁻⁸	COL3A1, MET, ITGB4, ITGA11, ITGA2, ITGA3, COL5A2, LAMB3, LAMA3, COMP, COL6A3, ITGB6, COL1A2, LAMC2, COL1A1, THBS2, COL11A1, FN1	
hsa04151	PI3K-Akt signaling pathway	21	20x10 ⁻⁷	COL3A1, MET, ITGA11, ITGB4, ITGA2, ITGA3, COL5A2, LAMB3, LAMA3, COMP, COL6A3, ITGB6, COL1A2, LAMC2, EFNA5, IL2RG, COL1A1, THBS2, ANGPT2, COL11A1, FN1	
hsa05146	Amoebiasis	11	4.4x10 ⁻⁶	IL1R2, LAMB3, LAMA3, COL3A1, COL1A2, LAMC2, COL1A1, SERPINB3, COL11A1, COL5A2, FN1	
hsa04974	Protein digestion and absorption	10	7.0x10 ⁻⁶	KCNN4, COL17A1, COL3A1, COL6A3, COL1A2, COL12A1, COL1A1, COL11A1, COL5A2, COL10A1	
hsa05412	Arrhythmogenic right ventricular cardiomyopathy	7	7.2x10 ⁻⁴	DSG2, ITGB6, ITGA11, ITGB4, LEF1, ITGA2, ITGA3	
hsa05202	Transcriptional misregulation in cancer	10	1.0x10 ⁻³	PLAT, IL1R2, MMP9, MET, ETV1, RUNX2, HPGD, HIST1H3H, PLAUI, HIST1H3I	
hsa05222	Small cell lung cancer	6	9.6x10 ⁻³	LAMB3, LAMA3, ITGA2, LAMC2, ITGA3, FN1	
hsa05230	Central carbon metabolism in cancer	5	1.6x10 ⁻²	SLC16A3, PKM, SLC2A1, MET, HK2	
hsa05410	Hypertrophic cardiomyopathy	5	3.1x10 ⁻²	ITGB6, ITGA11, ITGB4, ITGA2, ITGA3	
KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes; PDAC, pancreatic ductal adenocarcinoma.					

[illegible]

Figure 2. DEGs PPI network complex in PDAC. (A) All 386 DEG interaction networks.

thereby promoting cell carcinogenesis (27). Furthermore, the PI3K-Akt signaling pathway is important in the etiology of pancreatic cancer (28). Therefore, these signaling

pathways can promote the development of pancreatic cancer in a variety of ways, and may provide a new direction for the systematic treatment of pancreatic cancer.

B

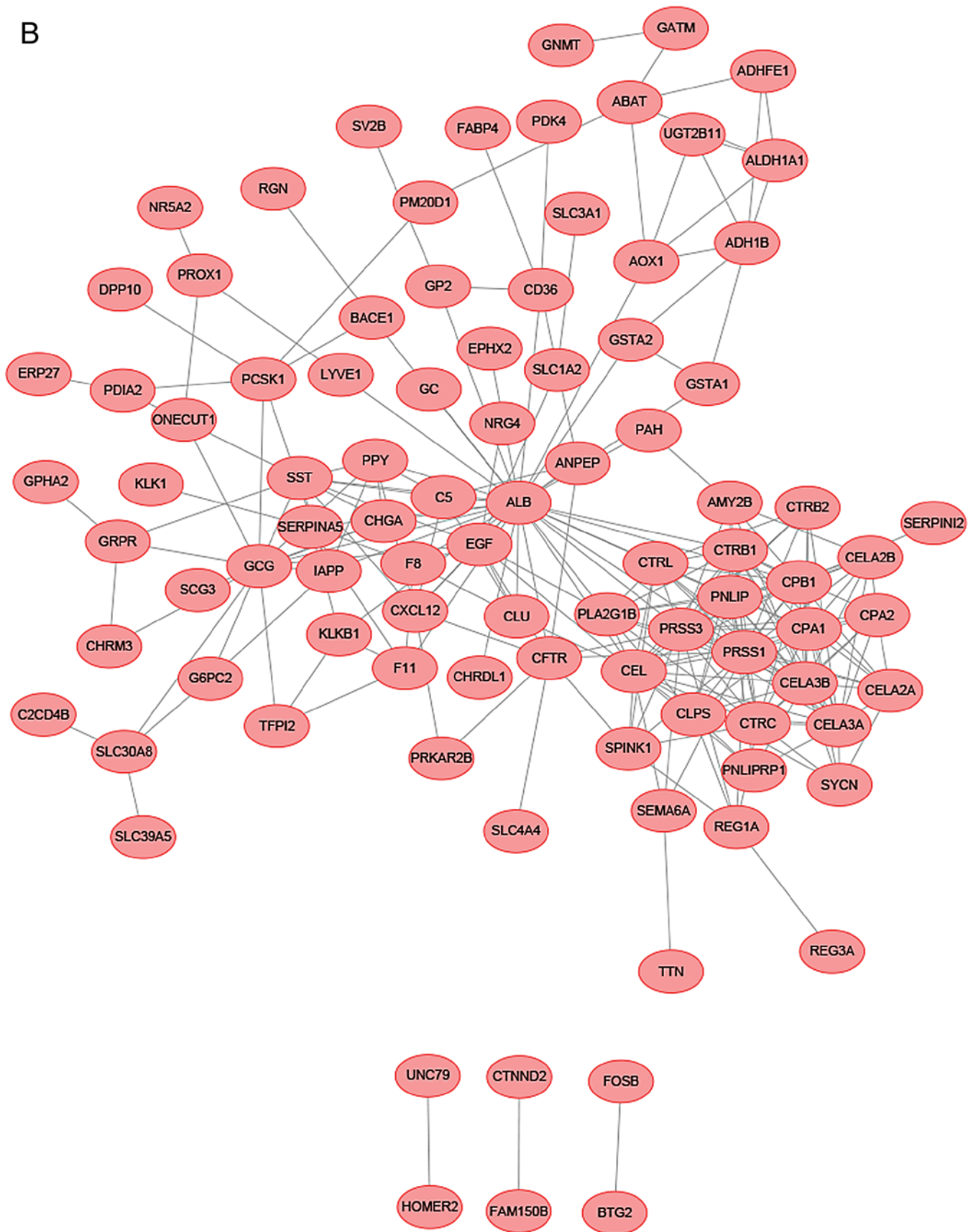


Figure 2. Continued. (B) The upregulated DEG interaction network.

In the current study, the top 10 degree hub genes identified in the PPI network were: CFTR, SLC7A2, CCL18,

PDK4, BAIAP2L1, ITGA3, CPA1, GPRC5A, STYK1 and ST6GALNAC1. CFTR was the highest scoring gene. The

C

Network graph showing interactions between numerous genes. The nodes are blue circles, and the edges are thin grey lines. The network is highly interconnected, with many nodes having multiple connections. The layout is somewhat circular, with a dense core of nodes and many peripheral nodes connected to the core. The gene names are in all caps and include various identifiers like HEPH, CP, NOX4, TFF1, AGR2, OLR1, ANKRD22, FBXO32, LMO7, SERPINB5, GPX2, CCL20, CXCL5, KCNN4, CCL18, NMU, ACSL5, CYP2C18, IL1RAP, IL1R2, FAM43A, PLAC8, CST1, CST2, ERO1L, GPX8, ANXA8L1, SRPX2, SYTL2, S100A14, S100A16, GABRP, ANO1, and many others. The network represents a complex biological system, likely related to the study of gene expression and protein interactions in the context of the provided text.

CFTR gene codes for the cystic fibrosis transmembrane conductance regulator protein, an important member of the ATP binding cassette transporter family (29). It serves an important role in anion regulation and tissue homeostasis of various epithelial cells, activates the cAMP channel and promotes chloride and bicarbonate secretion in the digestive system (30,31). A previous study revealed that increased expression of CFTR in drug-resistant prostate cancer tissues or cells that block CFTR can inhibit tumor cell viability and autophagy via the PI3K/Akt signaling pathway (32).

In CFTR knockout mice, mucosal barrier function was impaired, including tight junction disruption, which resulted in impaired tolerance to bacterial colonization and infection, abnormal innate and adaptive immune responses, and inflammation (33,34). It has been reported that CFTR is a negative regulator of the pro-inflammatory nuclear factor κ -light-chain-enhancer of activated B cells-mediated innate immune response, including interleukin-8, and evokes a positive feedback loop of cyclooxygenase 2-prostaglandin E2 in inflammation, and therefore, these factors may work

Table V. The enriched pathways of upregulated DEGs PPI network in PDAC.

A, Biological process			
Term	Description	Count	P-value
GO.0007586	Digestion	13	1.12×10^{-8}
GO.0065008	Regulation of biological quality	45	7.78×10^{-5}
GO.0044281	Small molecule metabolic process	36	1.85×10^{-4}
GO.0046903	Secretion	18	1.85×10^{-4}
GO.0002576	Platelet degranulation	7	6.08×10^{-3}
B, Molecular function			
Term	Description	Count	P-value
GO.0008236	Serine-type peptidase activity	13	2.61×10^{-8}
GO.0004252	Serine-type endopeptidase activity	12	2.83×10^{-7}
GO.0070011	Peptidase activity, acting on L-amino acid peptides	18	1.40×10^{-5}
GO.0008233	Peptidase activity	18	1.77×10^{-5}
GO.0004175	Endopeptidase activity	13	3.51×10^{-3}
C, Cellular component			
Term	Description	Count	P-value
GO.0005576	Extracellular region	76	2.57×10^{-15}
GO.0031988	Membrane-bounded vesicle	62	3.76×10^{-12}
GO.0044421	Extracellular region part	62	4.12×10^{-11}
GO.0005615	Extracellular space	35	8.18×10^{-11}
GO.0070062	Extracellular exosome	50	2.10×10^{-9}

DEGs, differentially expressed genes; PPI, protein-protein interactions; PDAC, pancreatic ductal adenocarcinoma.

together to promote tumorigenesis (35,36). The pancreas is a digestive organ that secretes a variety of substances to regulate the digestive fluids through exocrine and endocrine methods (37). At the same time, the abovementioned 10 hub genes can also regulate the development and progression of pancreatic cancer by regulating immune and inflammatory processes, protein glycosylation and energy metabolism which affect multiple signaling pathways (38-43). Therefore, these genes can be an important target for the precise treatment of pancreatic cancer.

For the upregulated DEGs, module analysis of the PPI network revealed that they were associated with pancreatic secretion signaling pathways and 'protein digestion and absorption' and 'lipid digestion and absorption' signaling pathways. Stimulation of the pancreas by secretagogues, including acetylcholine and cholecystokinin, results in intracellular Ca^{2+} signals, leading to the polarized secretion of enzymes (44). However, activation of the CFTR Cl^- channel and the CFTR-dependent $\text{Cl}^-/\text{HCO}_3^-$ exchange is responsible for cAMP-induced HCO_3^- secretion (44). The secretory function of the pancreas is directly associated with both protein

and lipid metabolism in the body, the disruption of which may lead to chronic inflammation of the pancreas, developing into pancreatic cancer (45).

The downregulated DEGs were associated with ECM-receptor interactions, focal adhesion and the PI3K-Akt signaling pathway (46). The ECM serves an important role in the morphogenesis of tissues and organs, and in the maintenance of cell and tissue structures and functions (47). These interactions lead to direct or indirect control of cell activity, including adhesion, migration, differentiation, proliferation, and apoptosis (48). Furthermore, the focal adhesion signaling pathway is the key signaling pathway of cell matrix adhesion, which serves an important role in cell movement, cell proliferation, cell differentiation, gene expression regulation and cell survival (49). The proliferation and metastasis of cancer cells depend on the regulation of this pathway (50,51). The PI3K-Akt signaling pathway serves as a bridge between extracellular signals and intracellular responses (52,53). Once activated, Akt phosphorylation can be involved in apoptosis, matrix control, important cellular processes, protein synthesis, metabolism and the cell cycle (54). The results obtained in the

Table VI. The enriched pathways of downregulated DEGs PPI network in PDAC.

A, Biological process			
Term	Description	Count	P-value
GO.0030198	Extracellular matrix organization	35	4.14x10 ⁻²⁴
GO.0022617	Extracellular matrix disassembly	20	1.55x10 ⁻¹⁷
GO.0030574	Collagen catabolic process	15	6.65x10 ⁻¹⁴
GO.0007155	Cell adhesion	32	4.45x10 ⁻⁹
GO.0001704	Formation of primary germ layer	13	5.25x10 ⁻⁹
B, Molecular function			
Term	Description	Count	P-value
GO.0005201	Extracellular matrix structural constituent	10	5.72x10 ⁻⁷
GO.0005518	Collagen binding	8	4.92x10 ⁻⁶
GO.0005539	Glycosaminoglycan binding	13	1.12x10 ⁻⁵
GO.0005515	Protein binding	66	1.16x10 ⁻⁴
GO.0004222	Metalloendopeptidase activity	9	1.94x10 ⁻⁴
C, Cellular component			
Term	Description	Count	P-value
GO.0005576	Extracellular region	100	7.19x10 ⁻²⁵
GO.0044421	Extracellular region part	91	2.70x10 ⁻²⁴
GO.0005615	Extracellular space	44	1.93x10 ⁻¹⁴
GO.0070062	Extracellular exosome	63	4.76x10 ⁻¹³
GO.0031012	Extracellular matrix	24	6.44x10 ⁻¹³
DEGs, differentially expressed genes; PPI, protein-protein interactions; PDAC, pancreatic ductal adenocarcinoma.			

current study suggest that pancreatic secretory dysfunction, the imbalance of ECM-associated signaling pathways and the PI3K-Akt signaling pathway may result in cell cycle disruption and metabolism-associated microenvironmental changes, which can trigger the development of pancreatic cancer.

In conclusion, the current study investigated the biological pathways involved in PDAC by providing a comprehensive bioinformatics map of DEGs. These DEGs are involved in the development and progression of PDAC and provide a basis for the effective study of the molecular mechanisms of pancreatic cancer. Further molecular biological experiments and animal studies are required to confirm the functions and roles of these DEGs in PDAC.

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Availability of data and materials

The datasets analyzed during the current study are available in the GSE28735 repository (www.ncbi.nlm.nih.gov/geo).

Authors' contributions

YH, YL and HW conceived of and designed the experiments. YH, YL, JG, CL and HZ performed the experiments. YH, YL and HW acquired, analyzed and interpreted the data and wrote the paper.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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