

Basic Gene Expression Characteristics of Tumor and Non-Tumor Tissues of Pancreatic ductal adenocarcinoma patients

Abstract: One of the deadly and the commonest malignant tumor is pancreatic ductal adenocarcinoma (PDAC). This study intended to clarify the promising prognostic and biomarker targets in PDAC using GSE78229 and GSE62452 data sets publicly accessible at NIH/NCBI Gene Expression Omnibus database. Utilizing GEOquery, Biobase, and gplots packages in R software 3.6 that is on the basis of expression analysis, we detect 221 differentially expressed genes (DEGs) of down regulation, whereas we find 207 up-regulated genes. The gene ontology (GO) and Kyoto Encyclopedia of Genes (KEGG) of pathway enrichments analyses of DEGs were studied. 28 KEGG pathways related with pancreatic ductal adenocarcinoma (PDAC) were detected, in which the endoplasmic reticulum protein processing pathway was noted to be significant. The following 21 hub genes were detected through NetworkAnalyst on the basis of protein-protein interaction (PPI) network by the STRING tool: UBC, RACK1, RPL18A, RPL8, RPS23, RPS3A, RPS6, RPL10, RPL7A, RPLP1, ACTB, RPL39, P4HB, PABPC1, HSP90B1, HSPA8, GAPDH, EXOC4, and JAK1. In the TCGA database, the quantification of expression of levels and survival probabilities were examined down and up-regulated DEGs and overall survival (OS) DEGs were investigated by Kaplan–Meier (KM) plotter (KM plotter). Moreover, the constructed study of protein-protein interactions and DAVID KEGG pathway enrichment study indicated as such ‘protein processing in endoplasmic reticulum’, ‘epstein-barr virus infection’, ‘platelet activation’, ‘ribosome’, ‘leukocyte’, ‘transendothelial migration’, and ‘protein digestion’ which had a close association with PDAC. Other hub genes discussed in this study, can be noted as promising targets for PDAC and related diseases diagnosis and treatment.

Keywords: biomarker; differentially expressed genes; pancreatic ductal adenocarcinoma; gene ontology pathway enrichment; cancer

1 Introduction

One of the most deadly cancer is pancreatic ductal adenocarcinoma (PDAC) which has a 5-year overall survival rate as 3% due to the diagnosis at a distant stage (1). There has been significant improvements in terms of treatments such as pancreatectomy, radiotherapy, adjuvant and neo-adjuvant chemotherapies and palliative care in the previous decades (2, 3). However, pancreatectomy still stays the most efficient treatment, specifically for initial phase pancreatic

cancer (4). Thus, an updated knowledge of simple and basic mechanism of pancreatic cancer is necessary for more useful and curable therapies and the advancement of patient survival.

Microarray has become an important tool in research of PDAC genes and target therapeutic drugs. Recent studies suggest an extensive gene expression analysis of PDAC and related diseases by reviewing expressed gene data sets through a comparison between tumor and normal tissues in the framework of PDAC (5,6).

Moreover, relative investigation of the different levels of expressed genes stays moderately constrained, and a dependable biomarker profile would be a need to develop new gene targets (7). The protein expression alterations in the advancement and growth of PDAC and related diseases require comprehensive analysis. Furthermore, the relations among the detected DEGs, specifically protein-protein interaction (PPI) networks and underlying signaling pathways should be clarified. Prospect molecular markers from such investigations can later be checked utilizing other methods to be used in early diagnosis of pancreatic cancers (8).

Wang et al., 2016 and Yang et al., 2016 recently performed experiments from pancreatic tissue from patients with pancreatic ductal adenocarcinoma of microarray gene-expression profiles (10, 11) “(data obtainable at NCBI GEO database , accession GSE78229 and GSE62452).”

By studying their hub nodes globally and between tumor and normal samples constructing PPI networks, the goal of this project is to study the pathway and genetic mechanisms of PDAC and related diseases growth and to come up with candidate biomarkers for diagnosis, therapeutic targets and predictions.

Earlier studies tackling pancreatic cancer and related diseases underlying biomarkers, due to the implicit evaluation of source and progenitor populations, need to support experimental studies with numerical analysis and statistical methods in addition to previous experiments on mice (11).

Traditional therapeutic alternatives, particularly chemotherapy, are not efficient enough fighting PDAC, and notwithstanding advancements over the last 15 years, the rate of survival has not increased and becoming one of the most lethal cancer type (12). Thus, constant efforts of the advancement of novel therapeutic alternatives is a need (13).

The advancement of high throughout sequencing has led the generation of countless gene expression profiles of pathological samples that are publicly reachable via the Gene Expression Omnibus (GEO) database (14,15). Whereas only a small part of these datasets has been studied, the different facets of the machinery of pancreatic tumor fast growth and resilience to therapies should be on focus. Using in-silico analysis, the deposited datasets are re-analyzed and used to offer beneficial outcomes for further examination. Throughout the re-examination procedure, differentially expressed genes (DEGs) are first detected with the underlying methods, and following the molecular and biological functions and pathways of the genes concerned are studied. Previously, various experimental studies are designed to understand pancreatic cancer (16,17). Even though the most of these research focused on the identification of the most significant genes experimentally, the tumor and non-tumor tissues paired comparison was usually not analyzed in-silico analysis. Thus, this study focused on two GEO datasets which contained paired samples such that tumor and adjacent non-tumor tissues, and the microarray expression data set was analyzed. The analysis provided the identification of the DEGs, and Gene Ontology (GO) and Kyoto Encyclopedia of Genes (KEGG) pathway enrichment analysis were

subsequently studied to examine the biological process, molecular function, and cellular component of the pathways and genes. Furthermore, a protein-protein interaction (PPI) network was created and a module analysis was explored to study the hub genes in pancreatic cancer. This study may provide fresh knowledge into the mechanism of pancreatic cancer expansion and its subsequent core genes, and the pathways concerned may assist as potential targets for the treatment of PDAC.

2 Materials and Methods

2.1. Preprocessing of the data set

The publicly accessible data set of gene expression from pancreatic tumor and normal samples were pull out from the GEO database with GSE62452 and GSE78229 which the affymetrix gene-expression data of these 50 samples were also included in the previous submission as GEO accession number GSE62452 (9,10). Genomic information ranging from gene sequences to protein structure predictions were obtained. The combined dataset contains a total of expression of 33,297 probes of in total 111 samples i.e., 50 tumor and 61 adjacent non-tumor tissue.

Utilizing the GEOquery package in Bioconductor subsequent conventional procedures in R studio, the datasets are studied (18). The list of other packages we utilized in R studio are Biobase, biomaRT, gplots, and ggplot2 packages (19–21). To calculate the adjusted *p value* and avoid Type I errors, we used Bejamini-Hochberg Procedure to rectify multiple testing. To adjust the statistical tests locally, hypergeometric model was performed for both of the down-regulated and up-regulated DEGs and false discovery rate (FDR) were estimated (22,23).

2.2. Gene expression data and analysis codes

Analysis were performed in the R programming language. Codes and data analysis of the GSE datasets can be reached at Github repository. Before conducting the analyses, the genes with very low number of reads and low quality reads were filtered out and the rest of the gene expression values converted to base-2 logarithmic scale using R language. We compared specimens into two groups provided that pancreatic tumor and non-tumor tissues. The data set was normalized by calculating the means of the samples of paired ones in R statistical environment. The analysis on paired samples was conducted as computing fold-change (biological significance) difference between the means of the categories. This study used a widely performed statistical model which is the t-distribution and its versions. Given the noisiness of the data i.e. the difference in averages between samples divided by the standard deviation, t-test compares the discrepancy of the mean gene expression levels between the two samples. Biomart package is utilized to annotate probes to official gene symbols. In order to detect DEGs, the converted gene symbols are filtered according to *p value* and fold change criterion. We stress statistical significance using *t-test* by taking *p value* cutoff 0.05 and $\log_2|\text{fold cut-off}| > 5$ and identify down and up-regulated DEGs between each category.

Although statistical methods to correct for multiple comparisons have been relevant for a great deal of time such as Bonferroni correction, vast majority of these methods are not suitable to analyze gene expression data sets (24).

2.3. Clustering analysis and Validation of Differentially expressed genes

Gene expression values were pull out for each sample and then converted to base-2 logarithmic scale by utilizing GEOquery package in Bioconductor in R language. The study utilized gplots and ggplots2 packages of R to construct heatmaps of DEGs with heatmap.2 function and barplots of GO pathways with ggplot function. The expression pattern of DEGs in pancreatic tumor and adjacent non-tumor tissues is validated via clustering analysis of DEGs.

2.4. GO and KEGG enrichment analysis of the pathways

Prior to gene expression measurements of annotations for down-regulated and up-regulated and DEGs, probe IDs are mapped to the official gene symbols and gene names using Biomart package in R language. Subsequently, the DEGs were characterized by their biological processes, molecular functions, and cellular components of GO and DAVID 6.8 (www.david.ncifcrf.gov) enrichments which stands for Visualization and Integrated Discovery (25). All categorized genes were carefully reviewed, and then parts such as Universal Protein source and physical properties GO and annotation types were retrieved using DAVID and KEGG Kyoto Encyclopedia Genes and Genomes (26). We then compared the results of DAVID with NetworkAnalyst enrichments performed with KEGG (27).

2.5. The protein-protein interaction (PPI) network

NetworkAnalyst (<https://www.networkanalyst.ca/>), publicly reachable on the web, offers analysis of PPI networks for single gene lists and expression values using STRING Interactome (28). For broad examination of the regulatory mechanisms in PDAC and related diseases, down and up-regulated DEGs of pancreatic tumors and normal tissues are used to construct a human PPI network. The core genes of the network detected with previously reported GO classification and enrichment.

2.6. Survival analysis

In reference to the database of TCGA, Ualcan (<http://ualcan.path.uab.edu/index.html>) (29) was utilized to perform survival analysis. Kaplan–Meier (KM) plotter survival analysis was carried out using down and up DEGs relying on the gene expression values in PDAC. In contrast to non-tumor tissues, gene expression levels presents important individual differences in tumor tissues. Low expression level shows the transcripts per million value (TPM) is equal or below the upper quartile whereas high expression level shows the TPM is above the upper quartile.

3 Results

3.1. Experimental data analysis

With gene expression result of the microarray expression datasets, we detect differentially expressed genes (DEGs) in total 428 genes from pancreatic tumor and non-tumor tissues which was shown with volcano plot (Figure 1). We find the down-regulated and up-regulated DEGs of pancreatic tumor and normal cell comparison. The expression values were pull out, and a heatmap was created to show the tumor and normal tissues discrepancy (Figure 2). DEGs were

selected with common t test, and labelled with $\log_2|\text{fold change}| > 5$ and $p < 0.05$. Here, the analysis detected 207 differentially expressed genes of up regulation, whereas it found 221 down-regulated gene.

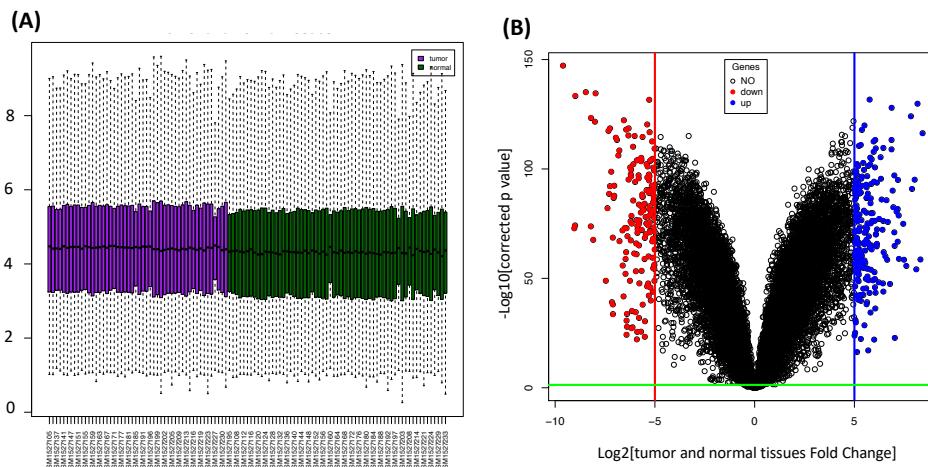


Figure 1 (A) The boxplot shows gene expression of each sample of the raw data without normalization. (B) Plots displaying the gene expression discrepancy in pancreatic tumor and normal tissues comparison. Black illustrates no change (NO), red illustrates down-regulated (Down), and blue illustrates up-regulated (Up) DEGs, FC, fold change.

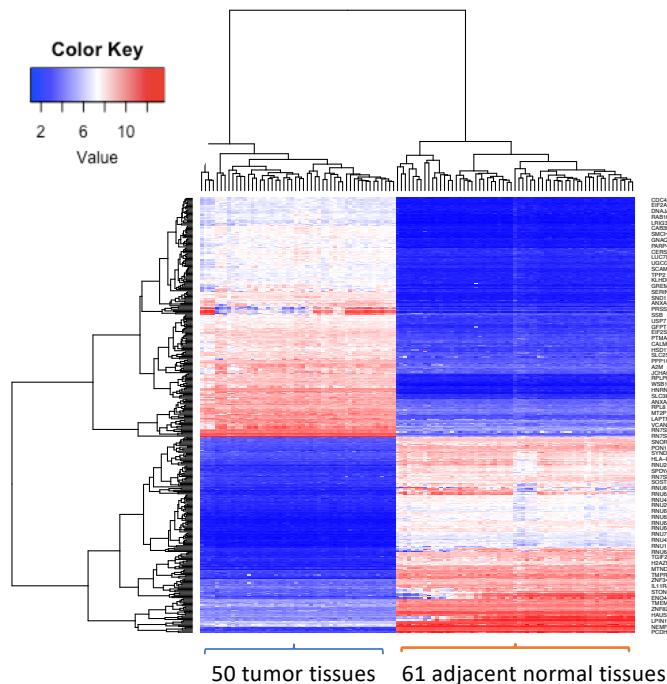


Figure 2 Heatmap demonstrates the top 60 differentially expressed genes (DEGs) in PDAC tumor and non-tumor tissues. Each columns present samples, and rows present official gene symbols. The gene expression values are converted base-2 logarithmic. The heatmap represents 30 downregulated genes (blue) and 30 upregulated genes (red). The gradual color varying from blue to red represents the ranging from down to up-regulated DEGs.

3.2 GO enrichment and KEGG pathways

The functions of the DEGs were categorized into the groups as the following: 'Biological Process', 'Molecular Function', 'Cellular Component' as is shown in Figure 3.

Table 1 shows the significant enrichment of DEGs using biological processes (BP) translational initiation (GO:0006413), nuclear-transcribed mRNA catabolic process (GO:0000184), SRP-dependent cotranslational protein targeting to membrane (GO:0006614), negative regulation of apoptotic process (GO:0043066), and cell-cell adhesion (GO:0098609)

The significant enrichment of DEGs in molecular function (MF) contains protein binding (GO:0005515), cadherin binding involved in cell-cell adhesion (GO:0098641), poly(A) RNA binding (GO:0044822), structural constituent of ribosome (GO:0003735), and extracellular matrix structural constituent (GO:0005201). Lastly, the significant enrichments of GO terms in cellular component (CC) is resulted extracellular exosome (GO:0070062), focal adhesion (GO:0005925), extracellular matrix (GO:0031012), extracellular space (GO:0005615), and membrane (GO:0016020).

In Table 2, the top 15 GO terms of the up-regulated DEGs and the top 11 GO terms of the down-regulated DEGs were ranked according to the gene counts and p-value criterion. The upregulated DEGs mainly enriched were associated with nuclear-transcribed mRNA catabolic process, translational initiation, SRP-dependent cotranslational protein targeting to membrane, viral transcription, and translation in the 'biological process' group, whereas concerning the 'molecular function' group poly(A) RNA binding, protein binding, structural constituent of ribosome, cadherin binding involved in cell-cell adhesion, RNA binding were identified. Furthermore, in the 'cellular component' gene ontology enrichment analysis revealed extracellular exosome, extracellular matrix, focal adhesion, membrane, and cytosolic large ribosomal subunit pathways. The downregulated DEGs that were basically enriched were associated with radial glia guided migration of Purkinje cell, striatum development, dephosphorylation, pre-pulse inhibition, and axonogenesis in the 'biological process' group. In the 'molecular function' gene ontology enrichment revealed cation channel activity. The enriched down-regulated DEGs were also associated with postsynaptic density, neuronal cell body, extracellular space, dendritic spine, and neuron projection regarding the 'cellular component' group.

These outcomes demonstrated that the most of the DEGs were significantly enriched in processes of vital cell organizations and functions, including extracellular matrix associated proteins, extracellular exosome formation, extracellular matrix organization, extracellular space and extracellular region.

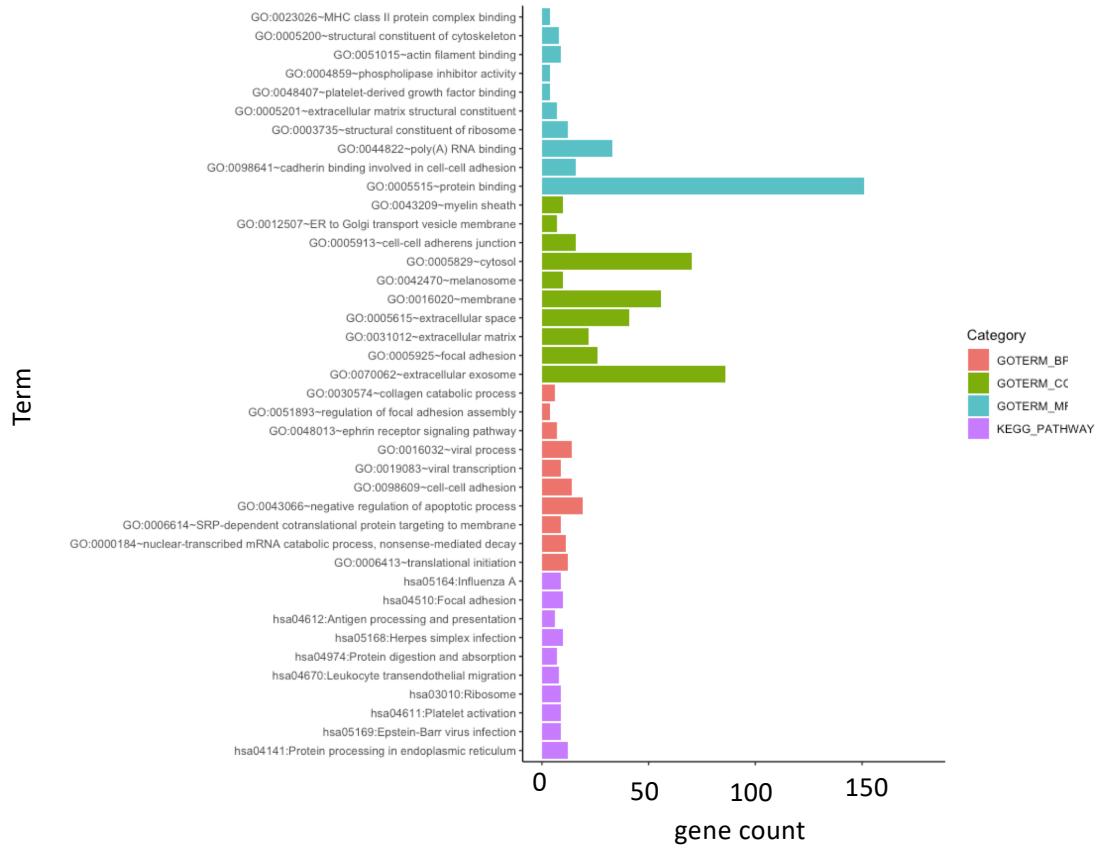


Figure 3. A bar plot of the DEGs 30 top gene ontology (GO) enrichments regarding biological processes (BP), cellular component (CC), molecular function (MF) is shown by colors of red, green, and blue respectively. The top 10 KEGG pathway enrichments of the DEGs is shown by the purple bars. The bars on the x-axis represented gene counts.

KEGG pathway study results revealed in which these DEGs were considerably enriched in protein processing in endoplasmic reticulum (hsa04141), Epstein-Barr virus infection (hsa05169), platelet activation (hsa04611), ribosome (hsa03010), leukocyte transendothelial migration (hsa03010). Among these pathways, endoplasmic reticulum protein processing pathway might have vital influence on multiple protein process which also has a role was aberrant in this disease.

Table 1. Gene expression data set retrieved with top significant pathways GO enrichment analysis of the DEGs in PDAC.

Category	Term	Count	p-value	Genes
GOTERM_BP	GO:0006413~translational initiation	12	3.05E-06	RPL7A, EIF2S3, RPL10, RPL18A, RPLP1, RPS6, RPS3A, PABPC1, RPL8, RPL39, EIF1, RPS23
GOTERM_BP	GO:0000184~nuclear-transcribed mRNA catabolic process, nonsense-mediated	11	5.74E-06	RPL7A, RPL10, RPL18A, PPP2R1A, RPLP1, RPS6, RPS3A, PABPC1, RPL8, RPL39, RPS23

	decay			
GOTERM_BP	GO:0006614~SRP-dependent cotranslational protein targeting to membrane	9	4.58E-05	RPL7A, RPL10, RPL18A, RPLP1, RPS6, RPS3A, RPL8, RPL39, RPS23
GOTERM_BP	GO:0043066~negative regulation of apoptotic process	19	6.12E-05	CD74, ANXA1, DUSP1, PRKDC, ANXA4, RPS6, ANXA5, RPS3A, SOD2, MT3, HSP90B1, NME1-NME2, GREM1, GOLPH3, UBC, MDM2, PDCD4, PDE3A, ANGPTL4
GOTERM_BP	GO:0098609~cell-cell adhesion	14	1.01E-04	YWHAE, RAB1A, HSPA8, SND1, RAB10, LIMA1, RPL7A, MYO1B, EIF2S3, SLK, KIF5B, PRDX1, RACK1, SPTBN1
GOTERM_MF	GO:0005515~protein binding	151	1.11E-06	
GOTERM_MF	GO:0098641~cadherin binding involved in cell-cell adhesion	16	6.76E-06	YWHAE, RAB1A, HSPA8, ANXA1, SND1, RAB10, LIMA1, RPL7A, MYO1B, EIF2S3, SLK, KIF5B, PRDX1, RACK1, CTNNA2, SPTBN1
GOTERM_MF	GO:0044822~poly(A) RNA binding	33	2.92E-05	YWHAE, RPL10, PRKDC, RPL8, RPL7A, PPP1CC, RPL18A, PRDX1, UBC, RACK1, DHX37, TNPO1, SPTBN1, HSPA8, DDX18, SSB, RPS6, RPS3A, HLA-A, DDX50, QKI, SND1, EIF1, CNOT1, HNRNPH1, SUB1, HNRNPA2B1, LUC7L3, SRSF5, PABPC1, P4HB, SLC25A5, RPS23
GOTERM_MF	GO:0003735~structural constituent of ribosome	12	1.70E-04	RPL7A, RPLP0P6, RPL10, RPL18A, RPLP1, RPS6, RPS3A, SLC25A5, RPL8, RPL39, RPS23, SLC25A6
GOTERM_MF	GO:0005201~extracellular matrix structural constituent	7	2.41E-04	COL1A1, COL3A1, VCAN, COL1A2, BGN, LAMB1, FBN1
GOTERM_CC	GO:0070062~extracellular exosome	86	3.56E-15	
GOTERM_CC	GO:0005925~focal adhesion	26	4.79E-11	YWHAE, RPLP1, RPL8, ACTB, ACTG1, HSP90B1, CORO1C, LIMA1, RPL7A, PPP1CC, B2M, JAK1, ACTR3, HSPA8, ANXA1, ANXA5, ADAM10, MSN, RPS3A, RAB10, MMP14, ARPC2, VIM, PABPC1, P4HB, BCAR1
GOTERM_CC	GO:0031012~extracellular matrix	22	2.79E-10	HSPA8, MMP7, PRKDC, BGN, LAMB1, RPS3A, ACTG1, HSP90B1, COL1A1, COL3A1, MMP14, VCAN, SFRP2, COL1A2, PRDX1, VIM, P4HB, SLC25A5, GAPDH, PRSS2, SLC25A6, FBN1
GOTERM_CC	GO:0005615~extracellular space	41	5.77E-07	
GOTERM_CC	GO:0016020~membrane	56	8.46E-07	
KEGG_PATHWAY	hsa04141:Protein processing in endoplasmic reticulum	12	3.08E-04	EDEM3, HSPA8, LMAN1, TRAM1, EIF2AK1, SEL1L, DNAJA2, DNAJC10, UBQLN1, SSR1, P4HB, HSP90B1
KEGG_PATHWAY	hsa05169:Epstein-Barr virus infection	9	0.00198029	USP7, MDM2, PLCG2, HLA-DRA, TNFAIP3, HLA-A, VIM, HLA-G, JAK1
KEGG_PATHWAY	hsa04611:Platelet activation	9	0.00294902	COL1A1, PPP1CC, COL3A1, COL1A2, ROCK1, GNAQ, PLCG2, ACTB, ACTG1
KEGG_PATHWAY	hsa03010:Ribosome	9	0.00389219	RPL7A, RPL10, RPL18A, RPLP1, RPS6, RPS3A, RPL8, RPL39, RPS23
KEGG_PATHWAY	hsa04670:Leukocyte transendothelial migration	8	0.00567818	ROCK1, PECAM1, PLCG2, MSN, CTNNA2, ACTB, BCAR1, ACTG1

Abbreviations- gene ontology: GO; biological process: BP; cell component: CC; Kyoto Encyclopedia of Genes and Genomes: KEGG (by the p value)

Table 2. GO enrichment analyses of the DEGs from 50 tumor and 61 adjacent non-tumor tissue

of PDAC patients. A, Down-regulated

Category	Term/gene function	count	%	P-value	Genes
GOTERM_BP	GO:0021942~radial glia guided migration of Purkinje cell	2	1.0929	0.02125823	DAB1, CTNNA2
GOTERM_BP	GO:0021756~striatum development	2	1.0929	0.0461805	CNTNAP2, SLTRK5
GOTERM_BP	GO:0016311~dephosphorylation	3	1.6393	0.05261072	ALPP, PON1, LPIN1
GOTERM_BP	GO:0060134~prepulse inhibition	2	1.0929	0.05434804	FABP7, CTNNA2
GOTERM_BP	GO:0007409~axonogenesis	3	1.6393	0.06623069	SLTRK5, KERA, CTNNA2
GOTERM_MF	GO:0005261~cation channel activity	2	1.09290	0.09604912	CATSPER3, TRPM6
GOTERM_CC	GO:0014069~postsynaptic density	4	2.18579	0.04441086	DAB1, MAP1B, CTNNA2, MT3
GOTERM_CC	GO:0043025~neuronal cell body	5	2.73224	0.04615778	CNTNAP2, DAB1, KLHL14, FABP7, RACK1
GOTERM_CC	GO:0005615~extracellular space	11	6.01093	0.06080918	OLFM3, PON1, IFNK, KRT78, SOSTDC1, GAST, KERA, PXDNL, ANGPTL4, MT3, CPA4
GOTERM_CC	GO:0043197~dendritic spine	3	1.63934	0.06842851	TENM2, MAP1B, MT3
GOTERM_CC	GO:0043005~neuron projection	4	2.18579	0.08135679	TENM2, DAB1, KLHL14, STON2
KEGG_PATHWAY	hsa04670:Leukocyte transendothelial migration	3	1.63934	0.08903048	PLCG2, CTNNA2, BCAR1

B, Up-regulated

Category	Term/gene function	count	%	P-value	Genes
GOTERM_BP	GO:0000184~nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	16	6.80851	5.02E-12	RPL4, RPL30, RPL10, RPLP1, RPS6, RPL8, PNRC2, RPL7A, RPS25, RPL18A, PPP2R1A, PABPC1, RPL39, RPS24, RPS23, RPL19
GOTERM_BP	GO:0006413~translational initiation	16	6.80851	3.95E-11	RPL4, RPL30, RPL10, RPLP1, RPS6, RPL8, EIF1, RPL7A, RPS25, EIF2S3, RPL18A, PABPC1, RPL39, RPS24, RPS23, RPL19
GOTERM_BP	GO:0006614~SRP-dependent cotranslational protein targeting to membrane	13	5.53191	6.22E-10	RPL4, RPL30, RPL10, RPLP1, RPS6, RPL8, RPL7A, RPS25, RPL18A, RPL39, RPS24, RPS23, RPL19
GOTERM_BP	GO:0019083~viral transcription	13	5.53191	4.82E-09	RPL4, RPL30, RPL10, RPLP1, RPS6, RPL8, RPL7A, RPS25, RPL18A, RPL39, RPS24, RPS23, RPL19
GOTERM_BP	GO:0006412~translation	15	6.38298	1.08E-06	RPL4, RPL30, RPL10, RPLP1, RPS6, RPL8, RPL7A,

					RPS25, RPL18A, SLC25A5, RPL39, RPS24, SLC25A6, RPS23, RPL19	
GOTERM_MF	GO:0044822~poly(A) RNA binding	40	17.0213	7.38E-11		
GOTERM_MF	GO:0005515~protein binding	138	58.7234	1.59E-10		
GOTERM_MF	GO:0003735~structural constituent of ribosome	15	6.3830	1.41E-07	RPL4, RPL30, RPLP0P6, RPL10, RPLP1, RPS6, RPL8, RPL7A, RPL18A, SLC25A5, RPL39, RPS24, SLC25A6, RPS23, RPL19	
GOTERM_MF	GO:0098641~cadherin binding involved in cell-cell adhesion	16	6.8085	6.44E-07	YWHAE, RAB1A, HSPA8, ANXA1, SND1, RAB10, LIMA1, RPL7A, MYO1B, EIF2S3, SLK, EPCAM, KIF5B, PRDX1, RACK1, SPTBN1	
GOTERM_MF	GO:0003723~RNA binding	19	8.0851	2.89E-05	RPL4, DDX18, RPL30, SSB, RPL8, DDX50, QKI, HSP90B1, RPL7A, RPS25, RPL18A, HNRNPH1, HNRNPA2B1, HNRNPD, PDCD4, PABPC1, SRSF5, RPL39, RPL19	
GOTERM_CC	GO:0070062~extracellular exosome	90	38.2979	7.45E-25		
GOTERM_CC	GO:0031012~extracellular matrix	25	10.6383	6.89E-15	RPL30, DDX5, PRKDC, ACTG1, HSP90B1, PRDX1, PRSS2, HSPA8, MMP7, BGN, LAMB1, COL1A1, RPS25, COL3A1, MMP14, VCAN, SFRP2, COL1A2, CANX, VIM, P4HB, SLC25A5, GAPDH, FBN1, SLC25A6	
GOTERM_CC	GO:0005925~focal adhesion	27	11.4894	5.36E-14	YWHAE, RPL4, RPL30, RPLP1, RPL8, ACTB, ACTG1, HSP90B1, CORO1C, LIMA1, RPL7A, PPP1CC, B2M, JAK1, RPL19, ACTR3, HSPA8, ANXA1, ANXA5, ADAM10, MSN, RAB10, MMP14, ARPC2, VIM, PABPC1, P4HB	
GOTERM_CC	GO:0016020~membrane	60	25.5319	2.68E-12		
GOTERM_CC	GO:0022625~cytosolic large ribosomal subunit	10	4.2553	3.49E-08	RPL4, RPL7A, RPL30, RPLP0P6, RPL10, RPL18A, RPLP1, RPL8, RPL39, RPL19	
KEGG_PATHWAY	hsa03010:Ribosome	13	5.5319	2.38E-06	RPL4, RPL30, RPL10, RPLP1, RPS6, RPL8, RPL7A, RPS25, RPL18A, RPL39, RPS24, RPS23, RPL19	
KEGG_PATHWAY	hsa04141:Protein processing in endoplasmic reticulum	13	5.5319	2.25E-05	EDEM3, HSPA8, TRAM1, EIF2AK1, SEL1L, HSP90B1, LMAN1, CANX, DNAJA2, DNAJC10, UBQLN1, SSR1, P4HB	
KEGG_PATHWAY	hsa05169:Epstein-Barr virus infection	10	4.2553	1.87E-04	USP7, MDM2, HLA-B, PLCG2, HLA-DRA, TNFAIP3, HLA-A, VIM, HLA-G, JAK1	

KEGG_PATHWAY	hsa04612:Antigen processing and presentation	8	3.4042	2.56E-04	HSPA8, CD74, CANX, HLA-B, HLA-DRA, HLA-A, B2M, HLA-G	
KEGG_PATHWAY	hsa05168:Herpes simplex infection	11	4.6808	9.22E-04	CD74, PPP1CC, USP7, EIF2AK1, CSNK2B, HLA-B, HLA-DRA, HLA-A, SRSF5, HLA-G, JAK1	

3.4. The protein-protein interaction network and KEGG pathway enrichment

Figure 4 shows the PPI map between the set of input DEGs. The expressions of the nodes and their degree of connection were symbolized by green to purple and fields, respectively in the visualized networks.

The genes with the best 19 scores according to the *p value* is identified; these proteins also determines the functionality of the PPI network. The human PPI map was constructed to identify the hub proteins using the betweenness centrality matrix. UBC, RACK1, RPL18A, RPL8, RPS23, RPS3A, RPS6, RPL10, RPL7A, RPLP1, ACTB, RPL39, P4HB, PABPC1, HSP90B1, HSPA8, GAPDH, EXOC4, and JAK1 were detected as the most connected hub proteins (Figure 4, Tables 1 and 2B). Best scoring genes comprise with PPI network KEGG enrichment analysis represents involvement ribosome (hsa03010), Ubiquitin mediated proteolysis (hsa04120), protein processing in endoplasmic reticulum (hsa04141), pathways in cancer (hsa05200). The analysis observed that DAVID and PPI network KEGG enrichment analysis revealed protein processing in endoplasmic reticulum (hsa04141), epstein-Barr virus infection (hsa05169), platelet activation (hsa04611), ribosome (hsa03010), leukocyte transendothelial migration (hsa04670) pathways in common.

Table 3 Top 10 most excessive KEGG pathway enrichment analysis of global DEGs in pancreatic tumor and non-tumor tissues micro-array gene expression data set.

Term	Count	p-value	Genes
hsa04141:Protein processing in endoplasmic reticulum	12	3.08E-04	EDEM3, HSPA8, LMAN1, TRAM1, EIF2AK1, SEL1L, DNAJA2, DNAJC10, UBQLN1, SSR1, P4HB, HSP90B1
hsa05169:Epstein-Barr virus infection	9	0.00198029	USP7, MDM2, PLCG2, HLA-DRA, TNFAIP3, HLA-A, VIM, HLA-G, JAK1
hsa04611:Platelet activation	9	0.00294902	COL1A1, PPP1CC, COL3A1, COL1A2, ROCK1, GNAQ, PLCG2, ACTB, ACTG1
hsa03010:Ribosome	9	0.0038922	RPL7A, RPL10, RPL18A, RPLP1, RPS6, RPS3A, RPL8, RPL39, RPS23
hsa04670:Leukocyte transendothelial migration	8	0.00567818	ROCK1, PECAM1, PLCG2, MSN, CTNNA2, ACTB, BCAR1, ACTG1
hsa04974:Protein digestion and absorption	7	0.00597994	COL1A1, CPA2, COL3A1, CPA1, COL1A2, SLC38A2, PRSS2
hsa05168:Herpes simplex infection	10	0.00724955	CD74, PPP1CC, USP7, EIF2AK1, CSNK2B, HLA-DRA, HLA-A, SRSF5, HLA-G, JAK1

hsa04612:Antigen processing and presentation	6	0.01371483	HSPA8, CD74, HLA-DRA, HLA-A, B2M, HLA-G
hsa04510:Focal adhesion	10	0.01503298	COL1A1, PDGFRA, PPP1CC, COL3A1, COL1A2, ROCK1, LAMB1, ACTB, BCAR1, ACTG1
hsa05164:Influenza A	9	0.01624626	IVNS1ABP, HSPA8, EIF2AK1, HLA-DRA, NXT2, PRSS2, ACTB, JAK1, ACTG1

Figure 4

The human PPI network of DEGs constructed via NetworkAnalyst. Hub genes are shown in (A) the network displayed is Subnetwork 1 and (B) Subnetwork 2 protein-protein interaction network. The colors present the expression values of the nodes. The “green” colored nodes presents up-regulated DEGs whereas the “purple” indicates the down-regulated DEGs. The gradual color alteration represents the expression levels. The number of the edges where the nodes linked each other presents the “node degree”. The node sizes show hierarchy of the significant genes in terms of degree centrality i.e., the greater quantity of neighbors a node has.

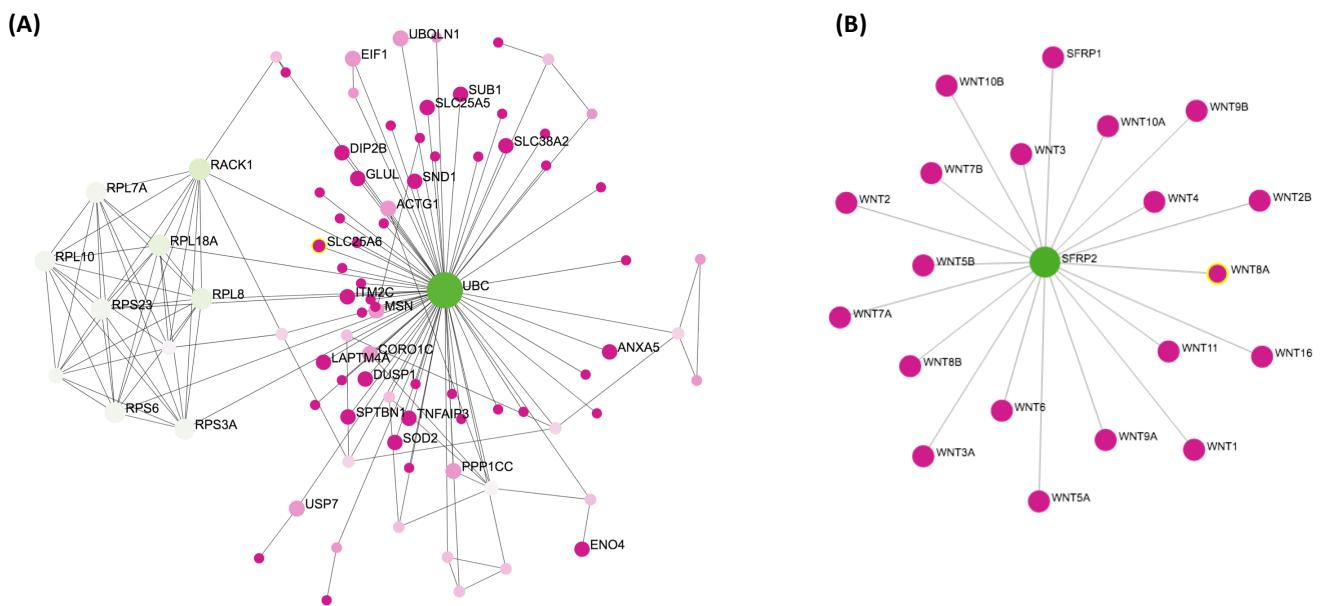


Figure 4 demonstrates the PPI network of all the DEGs in PDAC tumor and adjacent non-tumor tissues. Hub genes in Fig.4 can be listed as UBC and ribosomal protein (RP) gene family including both small (RPS) and large (RPL) subunits.

Table 4 Top 15 genes of PPI network of DEGs in PDAC tumor and non-tumor tissues gene expression data.

Gene ID	Genes	Node Degree	Betweenness centrality	Expression
7316	UBC	72	3370.87	6.89073

10399	RACK1	11	58.71	6.71587613
6142	RPL18A	10	56.87	6.71812216
6132	RPL8	10	56.87	6.14703757
6228	RPS23	9	38.72	5.46938802
6189	RPS3A	9	38.72	5.67086
6194	RPS6	9	38.72	5.43906883
6134	RPL10	9	0.78	7.22927072
6130	RPL7A	9	0.78	5.87863243
6176	RPLP1	8	0.5	4.95154703
60	ACTB	7	9.5	7.05993541
6170	RPL39	7	3.36	5.51250622
5034	P4HB	4	164	6.27703351
26986	PABPC1	4	20.22	5.54081207

3.7. The role of the endoplasmic reticulum protein processing pathway

The ultimate goal of this study due to its close association with pancreatic cancer and other related diseases through the significantly enriched pathways of DEGs is the analysis of the endoplasmic reticulum protein processing pathway.

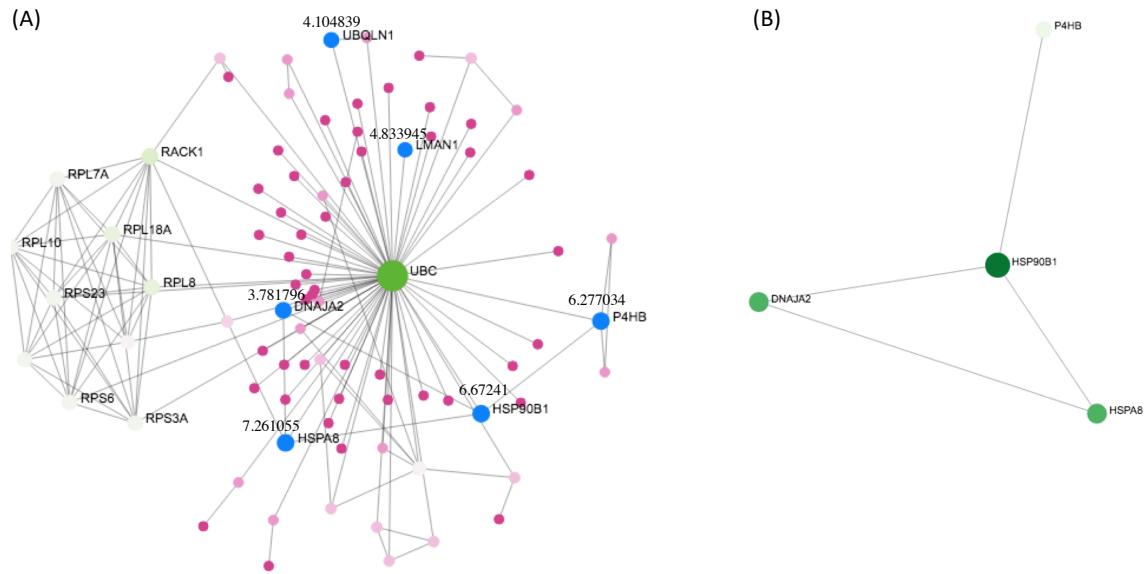
The endoplasmic reticulum (ER) is a cytoplasmic organelle in which excretory or membrane proteins are synthesized. Briefly, ER stress is a disparity within the protein folding capacity of ER and its protein pack that results the collection of cranking proteins (30,31). ER stress has been considered to be engaged in most of the deformational diseases, such as Parkinson's and Alzheimer's diseases and some of the particular morbid unfolding proteins have also been detected (32,33). Based on the results, we hypothesize that the entire endoplasmic reticulum protein processing pathway might be unsettled in PDAC because of over-expression of ER-associated proteins

Figure 5. There were 12 DEGs specifically engaged in ER protein processing pathway, containing EDEM3, HSPA8, LMAN1, TRAM1, EIF2AK1, SEL1L, DNAJA2, DNAJC10, UBQLN1, SSR1, P4HB, HSP90B1 (Fig. 4 and Table 1 and 2B). We have performed the primary DEGs associated with ER protein processing pathway in Figure 5. We observed "endoplasmic reticulum protein processing pathway" genes in PPI network of all the DEGs.

Related genes with the DEGs of the dataset enriched with the ER protein processing pathway deciphered as new hub genes. In Figure 5A, hub genes of this pathway are selected based on expression values. HSPA8, HSP90B1, P4HB, LMAN1, UBQLN1, and DNAJA2 is the most significant genes regarding gene expression values. The most expressed HSP gene family also performs a key position which is demonstrated in the subnetwork 2 of the ER protein processing pathway. Heat Shock Protein 90 Beta Family Member 1 (HSP90B1) might be a gene that is associated with this pathway, folding and transforming molecular chaperones with key roles in organizing other proteins. The HSP90B1 protein is contained in the endoplasmic reticulum.

HSP90B1 expression is associated with several pathogenic conditions, including tumor formation. Other DEGs are in subnetwork 2 are listed as HSPA8, P4HB, and DNAJA2 in pancreatic tumor and normal tissues. These findings verify the vital duty of the ER protein processing pathway engaged in PDAC and related diseases treatment, proposing new molecular therapeutical targets to fundamental drug agents.

Figure 5 The human PPI network of DEGs of gene expression in pancreas tissues identified by NetworkAnalyst emphasizing the endoplasmic reticulum protein processing pathway associated genes are shown with “blue” nodes. (A) The numbers (bigger to slower) represent gene expression values of HSPA8, HSP90B1, P4HB, LMAN1, UBQLN1, and DNAJA2 respectively of subnetwork 1. (B) HPS gene family has the key genes HSP90B1 and HSPA8 of the subnetwork 2.

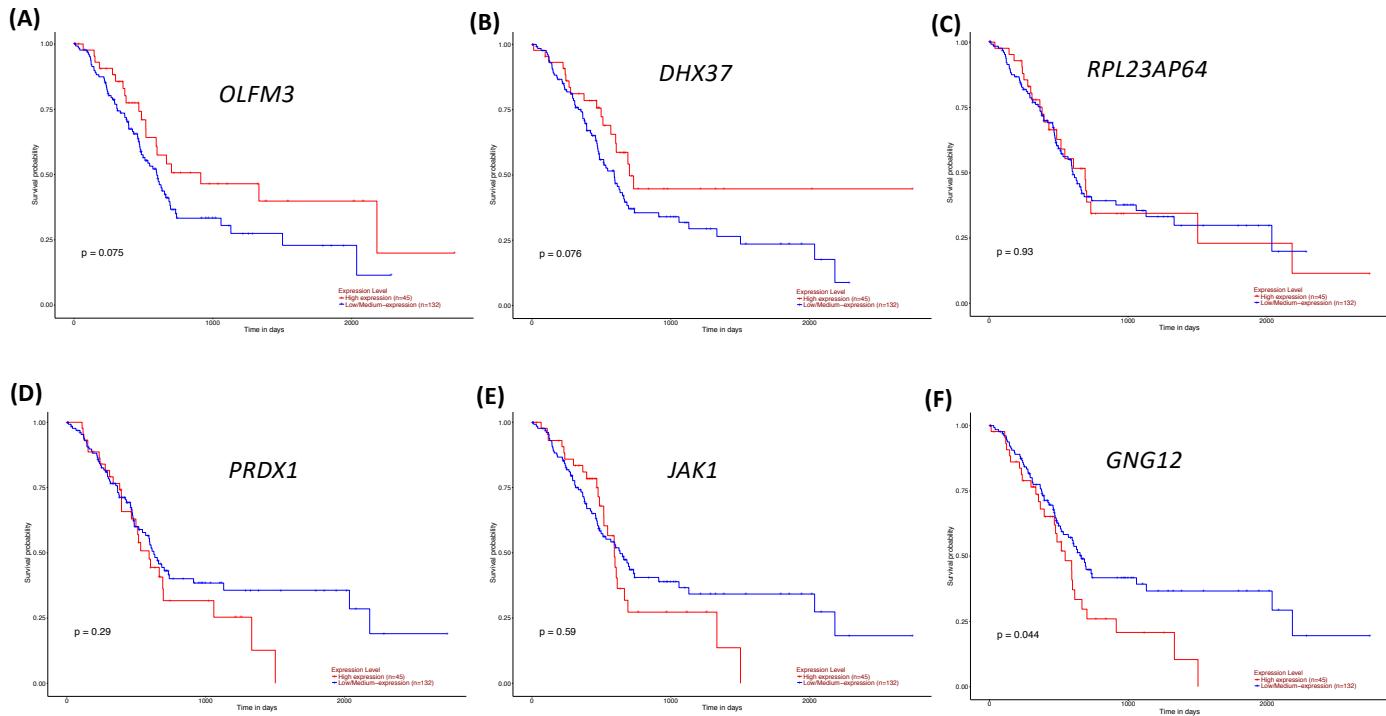


3.8 Survival Analysis

KM plotter was employed to anticipate the prognostic values of the 6 hub genes of down and up-regulated DEGs. Among the DEGs examined, the results displayed that the low expression of OLFM3, DHX37, and RLPL23AP64 were associated with worse overall survival (OS) for PDAC patients (Figure 6A-C). Additionally, high expression levels of PRDX1, JAK1, and GNG12 were associated with poor OS for PDAC patients (Figure 6D-F).

Figure 6 Prognostic value of six DEGs in PDAC patients

Effect of expression levels on PDAC patients of survival. Down-regulated DEGs (**A**) OLFM3 ($p = 0.075$), (**B**) DHX37 ($p = 0.076$), (**C**) RLPL23AP64 ($p = 0.93$) and up-regulated DEGs (**D**) PRDX1 ($p = 0.29$), (**E**) JAK1 ($p = 0.59$), and (**F**) GNG12 ($p = 0.044$).



4 Discussions

The prevalence of pancreatic ductal adenocarcinoma and the related survival rates have demonstrated a decreasing in tendency in the past years (1). One study showed that PDAC patients survive for only 4 months typically without therapies. Moreover, patients who undergo surgery and take required therapies the survival is not significantly increase. Thus, precise quick identification of PDAC and the advancement of powerful specific remedy is of fundamental significance. A recent research detected hub genes in PDAC that were stated to be of diagnostic significance (16).

In this project, combination of GSE78229 and GSE62452 datasets from patients with pancreatic ductal adenocarcinoma of microarray gene-expression profiles were comprehensively studied, holding gene expression of 50 tumor and 61 adjacent non-tumor tissues. Differentially expressed genes (DEGs) were only analyzed between tumor and normal tissues. 221 DEGs of down regulation were identified, whereas we found 207 up-regulated DEGs using R language. And further GO functional enrichments and KEGG pathway analyses were performed which showed locational and functional information of these DEGs. The outcomes of the GO enrichments shows that most of the DEGs were significantly enriched in processes of vital cell organizations and functions, including extracellular matrix associated proteins, extracellular exosome formation, extracellular matrix organization, extracellular space and extracellular region.

Furthermore, KEGG pathway analysis displayed that mostly the upregulated DEGs were entailed in ribosome (hsa03010), protein processing in endoplasmic reticulum (hsa04141), epstein-Barr virus infection (hsa05169), antigen processing and presentation (hsa04612), Herpes simplex infection (hsa05168). The DEGs involved in other pathways such as protein, digestion and absorption, focal adhesion, PI3K/Akt signaling pathway, pancreatic secretion,

oocyte meiosis, bacterial invasion of epithelial cells, and hippo signaling pathway might be of importance.

Recent studies have discovered an important role for 'ribosome' pathway genes involved in ribosome biogenesis in early pancreatic development (34). Moreover, recent research found that metabolic change is considered one of the features of cancer, especially the malfunction of pancreatic secretion. In pancreatic cancer, metabolic and functional changes are prominent in ribosomal and ER protein processing pathways (35,36).

Another recent review proposed the interaction of focal adhesions with the extracellular matrix might advance epithelial-mesenchymal transition (EMT), therefore foster cell carcinogenesis (37). Moreover, the PI3K/Akt signaling pathway (hsa04151) is the other significant pathway in the understanding of the pancreatic cancer (38).

Therefore, these pathways can facilitate to understand the expansion, metabolic and functional mechanisms of pancreatic cancer in several ways, and may supply a new regulation for the therapeutic and methodical treatment of pancreatic cancer.

This investigation further highlighted the endoplasmic reticulum protein processing pathway involving differentially expressed genes in a broad various kinds of human cancer (39). Furthermore, mutations employ in ER protein processing pathway genes and many related pathway genes provide pancreatic ductal adenocarcinoma carcinogenesis. In addition to their standard roles, ER protein processing pathway further rules metabolism characteristics of aggregation of misfolded proteins in the ER causes ER stress and activates a signaling pathway called the unfolded protein response (UPR).

To obtain an in-depth understanding of these DEGs, this study analyzed the constructed PPI network and found that UBC, RACK1, RPL18A, RPL8, RPS23, RPS3A, RPS6, RPL10, RPL7A, RPLP1, ACTB, RPL39, P4HB, PABPC1, HSP90B1, HSPA8, GAPDH, EXOC4, and JAK1 were the hub genes, which may be critical to the molecular mechanisms underlying the development of pancreatic tumor and might thus serve as promising therapeutic targets. Ubiquitin-C (UBC) was detected as one of the hub genes with the highest degree of connectivity. One study revealed that UBC expression associates with increased patient survival in PDAC (40). UBC is a key gene that directly engages with other genes such as RACK1 and ribosomal protein gene family (RP), proposes that it might be a central component that leads to a bad prognosis of PDAC regulated by perineural invasion (41). UBC has a vital task in diseases comprising renal cancer and lung cancer.

The receptor for activated protein kinase C (RACK1) is a scaffold protein participated in numerous intracellular signal pathways (42). Previous research have found that RACK1 is closely linked to the progression of several cancer types, along with gastric cancer and hepatocellular carcinoma. Recently, proteomic analysis performed in rat models of PDAC revealed that RACK1 was up-regulated in the pancreatic tissues and cell lines (43). Although the exact role of RACK1 in human PDAC growth still remains unidentified, it was found that RACK1 was significantly up-regulated in human PDAC samples and cell lines (44).

Taken together, the results of the bioinformatics analysis of four GEO microarray datasets of PDAC indicated that ribosome (hsa03010) and protein processing in endoplasmic reticulum (hsa04141), participate in the onset and development of PDAC. The low-expression of OLFM3, DHX37, and RLPL23AP64 , as well as the overexpression of PRDX1, JAK1, and GNG12, were observably related to unsatisfactory survival effects in patients with PDAC. However, further studies need to be implemented to discover the molecular mechanisms and biological functions

of the DEGs, biological processes, cellular component, and molecular function, KEGG pathways to estimate whether they can serve as novel potential biomarkers or therapeutic targets in PDAC patients.

Additional studies is required for clinical lab confirmation of predicted proteins that are expressed in PDAC tumor and non-tumor datasets and to express at the developmental stage of pancreatic ductal adenocarcinoma. More research is needed in the field of cancer biology to detect pancreatic cancer and subset diseases at its early stage. This paper also emphasizes the importance of microarray experiment in comprehending pancreatic cancer and related diseases and approach to study several results of gene expression data, like differentially expressed genes analysis, pathway and process identification, and protein-protein interaction network study.

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