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

Abstract: One of the mortal 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 based on 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 and Genomes (KEGG) of pathway enrichments investigation of DEGs were studied. 28 KEGG pathways related to 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 based on 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 the 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; gene ontology pathway enrichment; pancreatic ductal adenocarcinoma; cancer; endoplasmic reticulum

1 Introduction

One of the most deadly cancer is pancreatic ductal adenocarcinoma (PDAC) which has a 5-year overall viability rate of 3% due to the diagnosis at a distant stage (1). There have been significant improvements in terms of treatments such as pancreatectomy, radiotherapy, adjuvant, and neoadjuvant chemotherapies, and palliative care in the previous decades (2, 3). However, pancreatectomy still stays the most efficient treatment, specifically for the initial phase of pancreatic cancer (4). Thus, updated knowledge of the 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 the 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 the early diagnosis of pancreatic cancers (8).

Wang et al. 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 has become one of the most lethal cancer types (12). Thus, constant efforts of the advancement of novel therapeutic alternatives are a need (13).

The generation of countless gene expression profiles of pathological samples has been directed by the advancement of high throughput sequencing that is 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 pathways, molecular and biological functions of the genes concerned are examined. Previously, various experimental studies are designed to understand pancreatic cancer (16,17). Even though most of this research concentrated on the detection of the significant genes experimentally, the tumor and non-tumor tissue paired comparison was hardly studied in-silico analysis. Thus, this study focused on two GEO datasets which contained paired samples such that tumor and adjacent non-tumor samples, and the microarray expression data set was analyzed. The investigation provided the detection of the DEGs, and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were subsequently studied to examine the biological process, cellular component, and molecular function, of the pathways and genes and proteins. Moreover, a protein-protein interaction (PPI) network was created and a module analysis was explored to study the core genes in PDAC. This study may offer fresh knowledge into the machinery of pancreatic tumor expansion involving its subsequent core genes. Further, the pathways concerned in this study may assist as promising targets for the therapies of PDAC.

2 Materials and Methods

2.1. The gene expression datasets

The publicly accessible data set of gene expression from pancreatic tumor and normal samples pulled 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 was 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 tissues. Utilizing the GEOquery package in Bioconductor subsequent conventional techniques in R studio, the gene expression datasets are studied (18). The list of other packages employed in this study is Biobase, biomaRT, gplots, and ggplot2 packages in R version 3.6.3 (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, a hypergeometric model was performed for both of the down-regulated and up-regulated DEGs, and false discovery rates (FDR) were estimated (22,23).

2.2. Gene expression data analysis codes

Analysis was performed in the R programming language. Scripts in R programming and data analysis of the GSE datasets can be reached at https://github.com/guven-code/bioinformaticsAnalysis_PDAC repository. Before conducting the analyses, the genes with the very low number of reads and low-quality reads were filtered out and the rest of the gene expression values transformed to a base-2 logarithmic scale. The study then compared specimens into two groups provided that pancreatic tumor and adjacent non-tumor tissues. The expression values were normalized by taking the averages of the samples. The analysis of paired samples was conducted as calculating fold-change difference between the averages of the categories. This study used a widely utilized statistical model which is the t-distribution and its versions. Provided the noisiness of the samples which is the variance in averages between samples divided by the standard deviation, t-test compares the differences of the mean expression levels between the two samples. Biomart package is utilized to annotate probes to official gene symbols. To detect DEGs, the converted gene symbols are filtered according to *p-value* and fold change criterion.

Statistical significance threshold is decided taking *p-value* cutoff 0.05 and log2|fold cut-off| > 5 to identify down and up-regulated DEGs between each category using t-test.

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

2.3. Clustering analysis and Validation of Differentially expressed genes

By utilizing GEOquery package in Bioconductor in R language, expression values pulled out for each sample and then transformed to base-2 logarithmic scale. The study utilized gplots and ggplots2 packages of R to construct heatmaps of DEGs with heatmap.2 function and bar plots of GO pathways with ggplot function. The gene expression pattern of DEGs in PDAC 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 categorized into GO annotations of biological processes, molecular functions, and cellular components via DAVID 6.8 (www.david.ncifcrf.gov) enrichments which stands for the Database for Annotation, Visualization and Integrated Discovery (25). Each annotation type was retrieved using DAVID and KEGG Kyoto Encyclopedia Genes and Genomes (26). All annotated pathways were carefully reviewed and further partitioned according to the characteristics of their biological and molecular meanings.

2.5. The protein-protein interaction (PPI) network

NetworkAnalyst (https://www.networkanalyst.ca/), publicly reachable, offers the study of the PPI networks for single gene lists and expression values utilizing STRING Interactome (27). For broad examination of the regulatory mechanisms in PDAC and related diseases, down and up-regulated DEGs of pancreatic tumor and normal tissues are employed to construct a human PPI network. The core genes of the network were detected with previously reported GO classification and enrichment. The results of DAVID are then compared with NetworkAnalyst enrichments performed with KEGG (28).

2.6. Survival analysis

In reference to the database of The Cancer Genome Atlas Program (TCGA) (29) was utilized to perform survival analysis via Kaplan–Meier (KM) plotter. The 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 present important individual differences in tumor tissues. Low expression level shows the transcripts per million value (TPM) is equal to 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 the gene expression result of the microarray expression datasets, we detect differentially expressed genes (DEGs) in a total of 428 genes from pancreatic tumor and non-tumor tissues which was shown with a volcano plot (Figure 1). We find the down-regulated and up-regulated DEGs of pancreatic tumor and normal tissues comparison. The expression values pulled 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 *p-value* < 0.05 and log2|fold change| > 5. Here, the examination detected 207 differentially expressed genes of up-regulation, whereas it found 221 down-regulated genes.

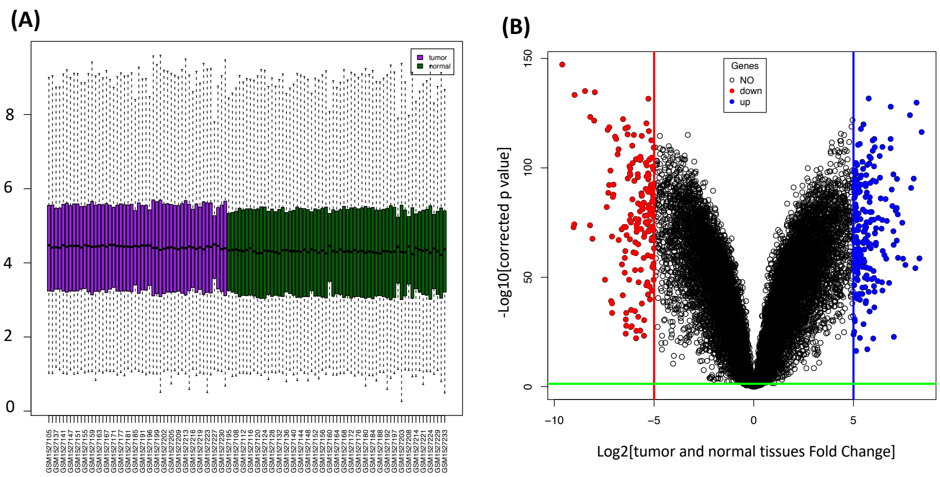


Figure (A) The boxplot shows gene expression values of each patient of the raw data without normalization. (B) Plots displaying the expression discrepancy in PDAC tumor and normal tissues comparison. Black illustrates no change (NO), red illustrates down-regulated (Down), and blue illustrates up-regulated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

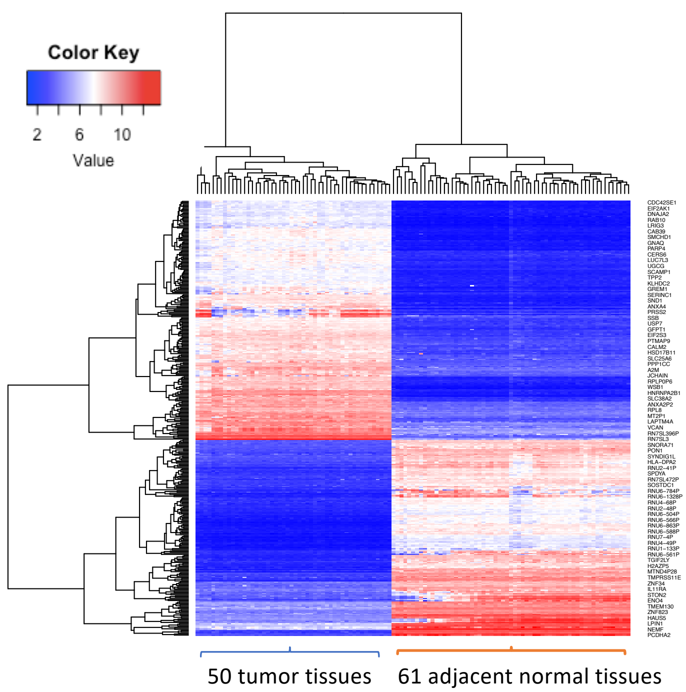


Figure 2 Heatmap demonstrates the top 60 DEGs in PDAC tumor and non-tumor tissues. Each columns present samples and rows present official gene symbols. The gene expression values are converted base-2 logarithmic. The heatmap represents 30 downregulated genes (blue) and 30 upregulated genes (red). The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

3.2 GO enrichment and KEGG pathways

The functional annotations of the DEGs were categorized into the groups as the following: 'Biological Process', 'Molecular Function', and 'Cellular Component' as is demonstrated 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 GO terms in molecular function (MF) comprises 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 11 GO terms of the down-regulated DEGs and the top 15 GO terms of the up-regulated DEGs and were ranked according to the gene counts and p-value criterion. The up-regulated 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, while 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 connected 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. The outcomes of GO annotations demonstrated that most of the DEGs were strongly 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.

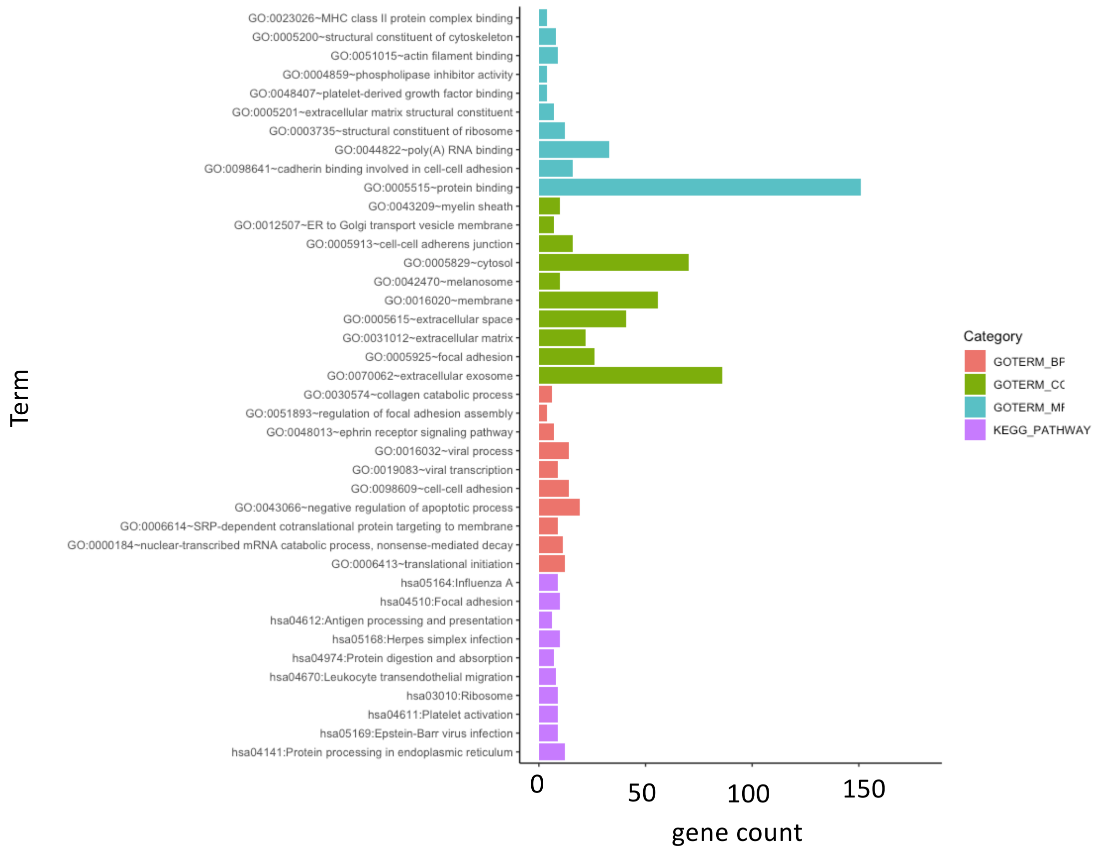


Figure 3. A bar plot of the DEGs the 30 top GO annotations regarding biological processes (BP), cellular component (CC), molecular function (MF) is shown by colors of red, green, and blue respectively. The top 10 KEGG pathway enrichments of the DEGs is shown by the purple bars. The bars on the x-axis 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 a 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 annotations 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 decay	11	5.74E-06	RPL7A, RPL10, RPL18A, PPP2R1A, RPLP1, RPS6, RPS3A, PABPC1, RPL8, RPL39, RPS23

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 annotation results of the DEGs from 50 tumor and 61 adjacent non-tumor tissues 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, SLITRK5
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	SLITRK5, 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* are identified; these proteins also determine the functionality of the PPI network. This network was constructed to detect 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 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 PDAC 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 was created via NetworkAnalyst. Hub genes are shown in the network displayed is (A) Subnetwork 1 and (B) Subnetwork 2 human PPI networks. The colors present the expression values of the nodes.
The "green" colored nodes present up-regulated DEGs whereas the "purple" indicates the down-regulated DEGs. The gradual color alteration presents the gene expression levels. The number of the edges where the nodes linked each other presents the "node degree". The node sizes show a hierarchy of the important genes in terms of degree centrality i.e., the greater quantity of neighbors a node has.

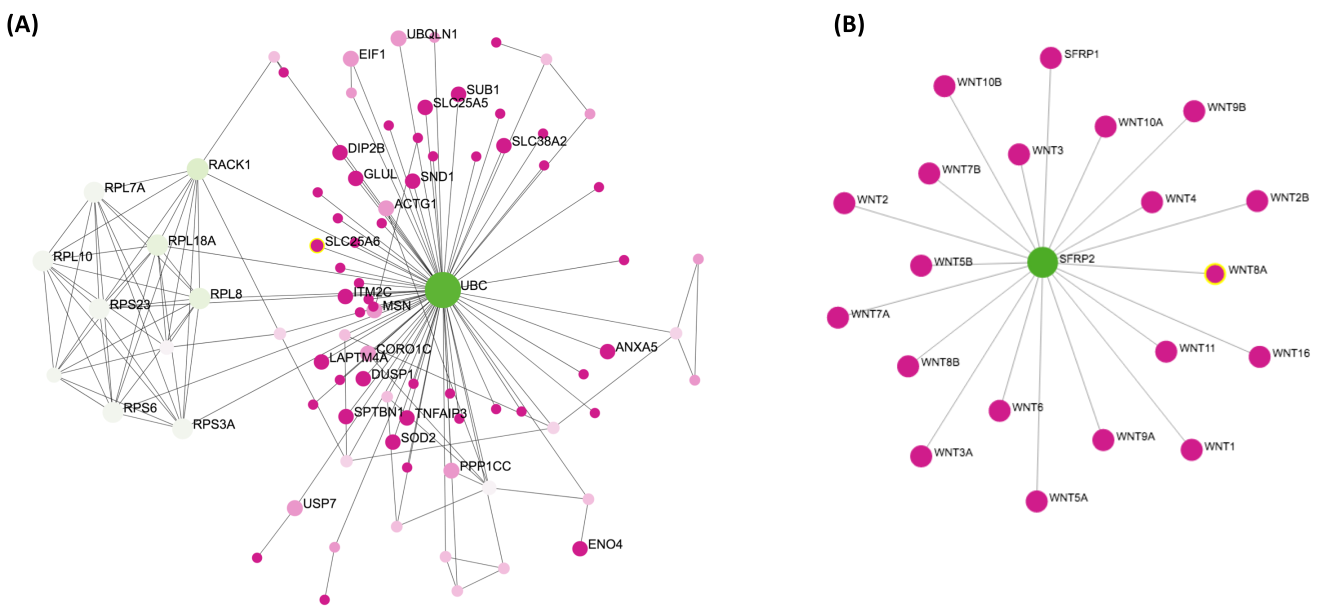


Figure 4 demonstrates the PPI network of DEGs in PDAC tumor and adjacent non-tumor tissues. Hub genes in Fig.4 can be listed as UBC and ribosomal protein (RP) gene family sub-setting both small (RPS) and large (RPL) subunits.

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

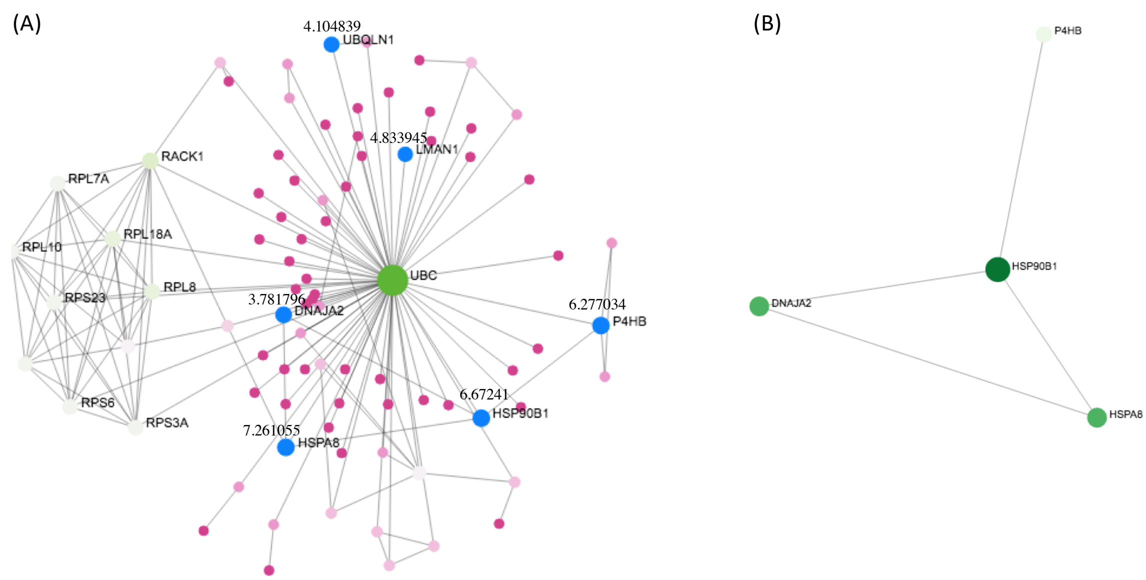
Gene ID	Genes	Node Degree	Betweenness centrality	Expression
7316	UBC	72	3370.87	6.89073

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

One of the goals of this study because of its strong connection with pancreatic cancer and other related diseases through the significantly enriched pathways of DEGs in the analysis of the “endoplasmic reticulum protein processing” pathway.The endoplasmic reticulum (ER) is one of cytoplasmic organelles whereupon excretory or membrane proteins are compounded. Shortly, ER stress is a disparity within the protein folding capacity of ER and its protein pack that results from the collection of crankling proteins (30,31). ER stress has been considered to be engaged in the majority of the deformational diseases, such as Parkinson’s and Alzheimer’s diseases and a few of the particular morbific unfolding proteins have also been detected (32,33). According to the results, we hypothesize that the entire endoplasmic reticulum protein processing pathway might be unsettled in PDAC by virtue 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 the PPI network of the entire DEGs. Related DEGs of the dataset are enriched with the ER protein processing pathway that was 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 are the most significant genes regarding gene expression values. The most expressed HSP gene family also performs a key position which is demonstrated in 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 updated molecular targets to the basic drug agents.

Figure 5 The PPI network of DEGs in PDAC 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.

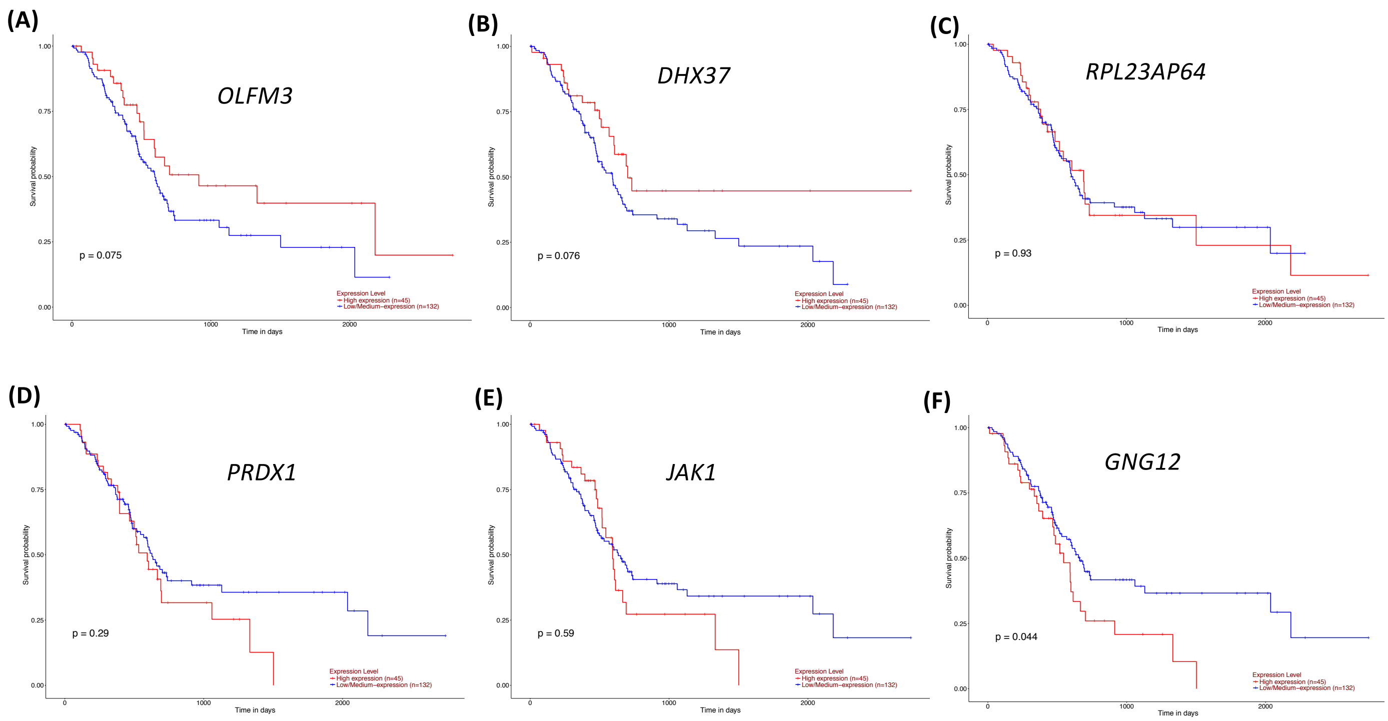


3.8 Survival Analysis

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 was related to worse overall survival (OS) for PDAC patients (Figure 6A-C). Additionally, over-expression levels of PRDX1, JAK1, and GNG12 were related to poor OS for PDAC patients (Figure 6D–F).

Figure 6 Prognostic values 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 decrease in tendency in the past years (1). One study showed that PDAC patients survive for only 4 months typically without therapies. Moreover, for patients who undergo surgery and take required therapies the survival is not significantly increased. Thus, precise quick identification of PDAC and the advancement of powerful specific remedies is of fundamental significance. Recent research detected hub genes in PDAC that were stated to be of diagnostic significance (16).

In this project, the combination of GSE78229 and GSE62452 datasets from patients with pancreatic ductal adenocarcinoma of microarray gene expression profiles were comprehensively studied, holding the expressions of 50 tumor and 61 adjacent non-tumor tissues. 221 differentially expressed genes (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 show that the vast majority 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.

In addition, KEGG pathway study resulted mostly in 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 are involved in other pathways such as protein, digestion and absorption, PI3K/Akt signaling pathway, focal adhesion, 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 alteration is regarded as one of the features of cancer, particularly the malfunction of pancreatic secretion. Metabolic and functional changes in ribosomal and ER protein processing pathways are evident in pancreatic cancer (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 PDAC (38).

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 endoplasmic reticulum which drives ER stress and then triggers the unfolded protein response (UPR) signaling pathway.

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 and biological mechanisms underlying the development of pancreatic tumor and may thus serve as promising therapeutic targets. Ubuiquitin-C (UBC) was detected as one of the core genes with the biggest 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.

RACK1 is a scaffold protein that is a receptor at the same time that initiates signal transduction of protein kinase C that is participated in most of the intracellular signal pathways (42). Earlier research has found that RACK1 is closely linked to the development and progression of several cancer types, along with gastric cancer and hepatocellular carcinoma (43). Although the exact involvement of RACK1 in human PDAC growth still stays unidentified, it was established that RACK1 was significantly up-regulated in human PDAC samples and cell lines (44, 45).

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 components, and molecular function, KEGG pathways to estimate whether they can serve as novel potential biomarkers or therapeutic targets in PDAC patients.

Additional studies are 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 their early stage. This paper also emphasizes the importance of microarray experiments 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.

Abbreviations:

DEGs: Differentially Expressed Genes
PDAC: Pancreatic Adenocarcinoma
DAVID: The Database for Annotation, Visualization and Integrated Discovery
KEGG: Kyoto Encyclopedia of Genes and Genomes
GEO: Gene Expression Omnibus
GO: Gene Ontology
ER: Endoplasmic Reticulum
PPI: Protein-protein Interaction
BP: Biological Process
MF: Molecular Function
CC: Cellular Component

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