

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

Abstract: One of the lethal and the commonest malignant tumor is pancreatic ductal adenocarcinoma (PDAC). The present study aimed to elucidate the potential biomarker and prognostic targets in PDAC using GSE78229 and GSE62452 data sets publicly reachable at NIH/NCBI Gene Expression Omnibus database. Using Biobase, GEOquery, gplots packages in R software 3.6 that is based on gene 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, may be used as potential 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 a significant tool in investigating pancreatic cancer 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 cells (5–7).

Moreover, relative investigation of the different level of expressed genes stays moderately constrained, and a dependable biomarker profile would be a need to develop new gene targets (8). The protein expression alterations in the advancement and growth of pancreatic ductal adenocarcinoma cancer 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 (9).

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 (12).

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 (13). Thus, constant efforts of the advancement of novel therapeutic alternatives is a need (14).

The advancement of high throughout sequencing has resulted in the generation of countless gene expression profiles of neoplasms that are publicly reachable via the Gene Expression Omnibus (GEO) database (15,16). Whereas only a small part of these datasets has been studied, the different facets of the mechanism of pancreatic tumor development and the resistance to treatments may be investigated. Using a bioinformatic analysis, the deposited datasets are re-analyzed and used to provide beneficial outcomes for subsequent examination. Throughout the re-analysis procedure, differentially expressed genes (DEGs) are first identified, and subsequently the molecular and biological functions and pathways of the genes concerned are studied. Several in-silico paired studies performed in pancreatic cancer previously (17,18). Although the majority of these studies only focused on the identification of the most significant genes mostly, the tumor and normal tissues paired comparison was usually not analyzed in those studies. Therefore, in the present study, two GEO datasets were selected, which contained paired tumor tissues and adjacent non-tumor tissues, and the microarray expression data set was analyzed. The analysis led to the identification of the DEGs, and Gene Ontology (GO) and pathway enrichment analysis were subsequently performed to explore the biological functions and pathways of these genes. Furthermore, a protein-protein interaction (PPI) network was constructed and a module analysis was performed to explore the hub genes in pancreatic cancer. The present study may provide novel understanding into the mechanism of pancreatic cancer

formation and its subsequent core genes, and the pathways involved may serve 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 (10,11). 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.

Using the GEOquery package in Bioconductor following conventional procedures in R studio, the datasets are studied (19). The other packages we used in R studio are as the following; Biobase, biomaRT and gplots packages (20,21). To estimate the adjusted *p value* and avoid Type I errors, we used Bejamini-Hochberg Procedure to correct multiple testing. In order to adapt the statistical tests locally, hypergeometric model was performed for both of the down-regulated and up-regulated DEGs in the pathway and gene ontology (GO) enrichment analysis, and false discovery rate (FDR) were computed (22,23).

2.2. Experimental data and analysis codes

Analysis were conducted in the R statistical environment. Sample codes and analysis of the GSE datasets can be found Github repository. Prior to the analyses, the low quality reads and genes with very low number of reads were removed and gene expression values converted to base-2 logarithmic scale using R language. We compared samples into two groups provided that pancreatic tumor and non-tumor tissues. The data set was normalized by computing the means of the samples of each group in R programming language. The process on separated samples which is grouped by categories was performed as computing fold-change (biological significance) difference between the means of the categories. A broadly performed statistical model is the t-distribution and its versions. A t-test compares the discrepancy of the average gene expression levels between the two samples or subgroups, given the noisiness of the data i.e. the difference in means between samples divided by the standard deviation. 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 highlight statistical significance performing *t-test* by taking *p value* cutoff 0.05 and $\log_2|\text{fold cut-off}| > 5$ to identify down and up-regulated DEGs between each category under study.

Despite the fact that, methods to correct for multiple comparisons have been applicable for a long time such as Bonferroni correction, most of these methods are not appropriate to analyze gene expression data sets (24).

2.3. Differentially expressed genes and clustering analysis

Using GEOquery package in Bioconductor, gene expression values were pull out for each sample and converted to base-2 logarithmic scale using R language. The study used gplots and

ggplots2 packages of R to create heatmaps of DEGs with heatmap.2 function and barplots of GO pathways with ggplot function. Clustering analysis of DEGs was done to match the expression pattern of DEGs in pancreatic tumor and adjacent non-tumor tissues.

2.4. GO terms and analysis of the pathway enrichments

Expression measurements annotations for up-regulated and down regulated DEGs for each group probes mapped to official gene symbols and gene names using Biomart package in R. All of the DEGs were characterized by their biological processes, molecular functions, and cellular components of GO and DAVID enrichments which stands for Visualization and Integrated Discovery (25). All classified genes were cautiously examined and further parts like the Universal Protein resource, and physical properties GO and annotation types were taken using DAVID and KEGG Kyoto Encyclopedia of 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, publicly accessible on the web, provides analysis of PPI networks for single gene lists using STRING Interactome (28). To comprehensively decipher the regulatory mechanisms in PDAC and related diseases, DEGs from pancreatic tumors and normal tissues were analyzed to form a PPI network and resulted core genes detected with previously reported GO classification and enrichment.

2.6. Survival analysis

In reference to the TCGA database, Ualcan (<http://ualcan.path.uab.edu/index.html>) (29) was utilized to conduct 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 **normal tissues** which was demonstrated in 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 cell discrepancy (Figure 2). DEGs were selected with common *t* test, and labelled with $\log_2|\text{fold change}| > 5$ and $p < 0.05$. Here, we detected 207 differentially expressed genes of up regulation, whereas we find 221 down-regulated gene.

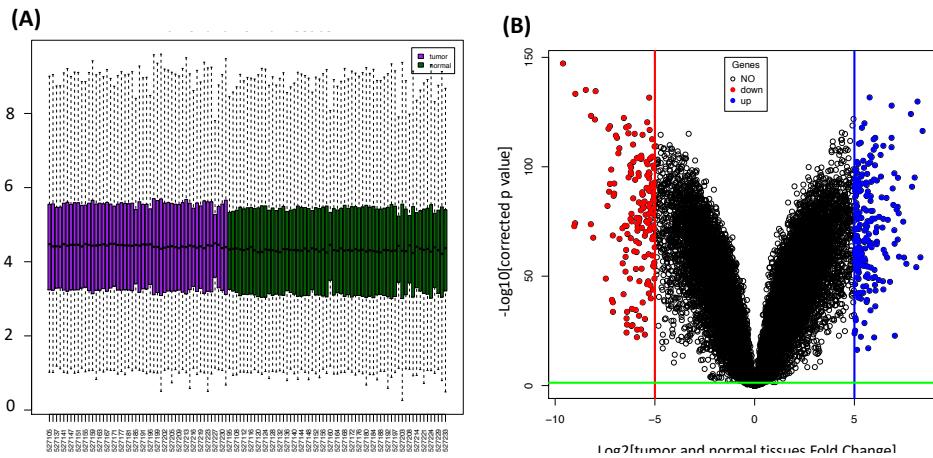


Figure 1 (A) The boxplot shows gene expression of each sample of the raw data without normalization. (B) Plots displaying the gene expression discrepancy in pancreatic tumor and normal tissues comparison. Black illustrates no change (NO), red illustrates down-regulated (Down), and blue illustrates up-regulated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

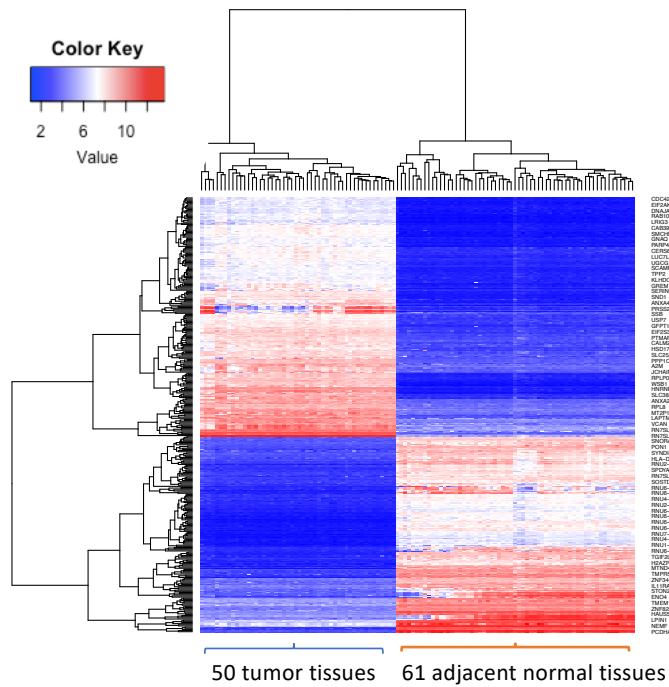


Figure 2 Heatmap demonstrates the top 60 differentially expressed genes (DEGs) in pancreatic tumor and normal tissues. Each column present samples, and rows present official gene symbols. Base-2 logarithmic values of the gene expression data are calculated. The heatmap presents 30 upregulated genes (red) and 30 downregulated genes (blue). The progressive color changing from blue to red represents the ranging from down to up-regulated DEGs.

3.2 Gene ontology enrichment and KEGG pathway analysis

The functions of the DEGs were classified into the groups as follows: '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). Finally, the significant enrichments GO terms in cellular component (CC) is revealed 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 that were primarily 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 with regard to 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 primarily 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 with regard to the 'cellular component' group.

These outcomes demonstrated that the majority of 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.

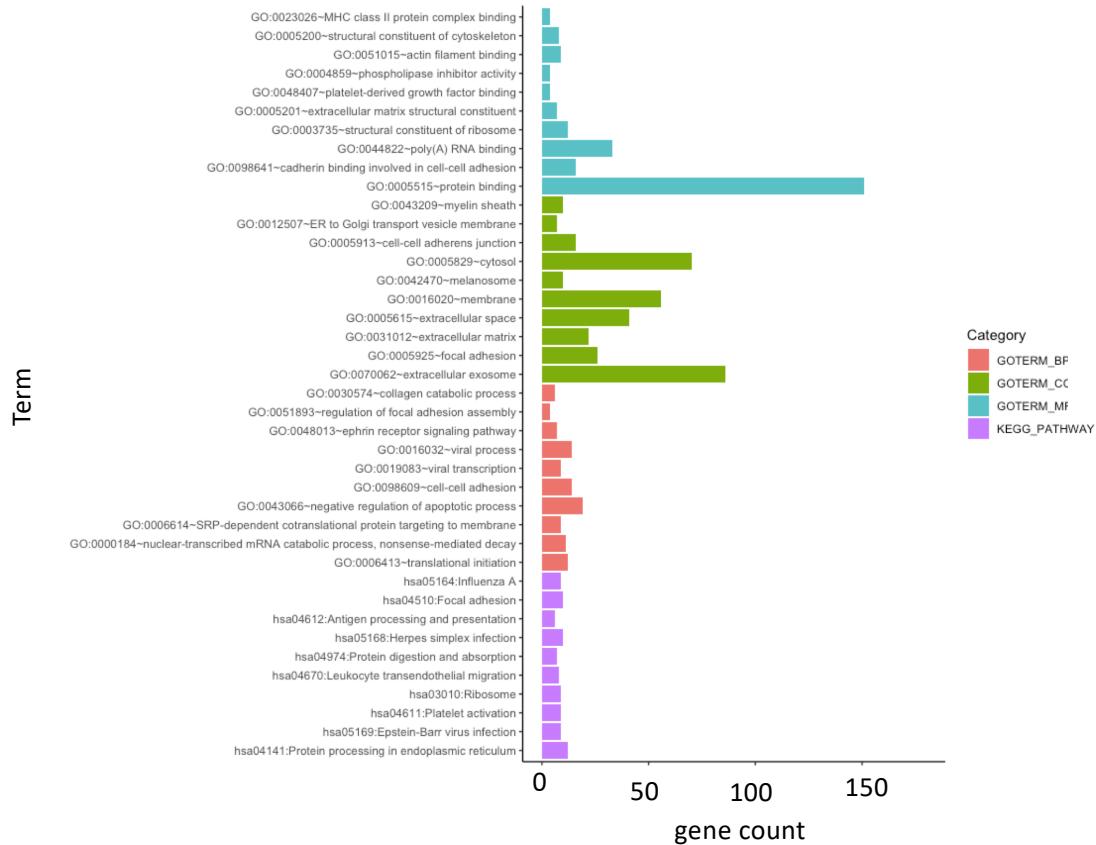


Figure 3. The significant enrichments of DEGs using 30 top gene ontology (GO) enrichments of each biological processes (BP), cellular component (CC), molecular function (MF) and 10 to KEGG pathways.

KEGG signaling pathway study outcomes demonstrated 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 functional enrichment analyses of differentially expressed genes of 50 tumor and 61

adjacent non-tumor tissue of PDAC. 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. Then the generic PPI map was used 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 identified as the most connected nodes (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 normal 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

Human PPI network of DEGs of gene expression in tumor and non-tumor pancreatic tissues identified by NetworkAnalyst. Hub genes are shown in (A) the network displayed is Subnetwork 1 and (B) Subnetwork 2 protein-protein interaction network. The colors represent the expressions of nodes. Specifically, “green” and “purple” indicate the nodes are up and down-regulated, respectively. The progressive color changing represents the expression levels. The areas of the nodes indicate the degrees that the nodes connect to others. The node sizes represent ranking of significant genes in terms of degree centrality i.e., the greater quantity of neighbors a node has.

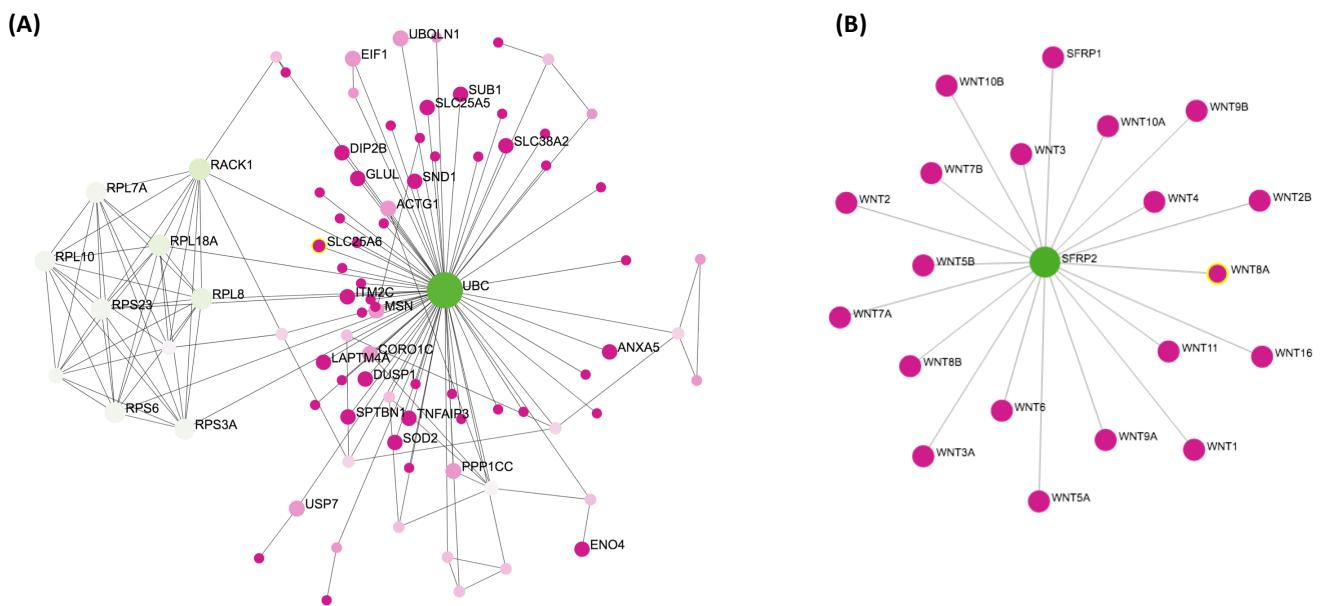


Fig.4 demonstrates PPI network of DEGs in pancreatic ductal tumor and adjacent non-tumor tissues of all the DEGs . Hub genes in Fig.4 can be listed as UBC and ribosomal protein (RP) gene family including both small (RPS) and large (RPL) subunits.

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

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

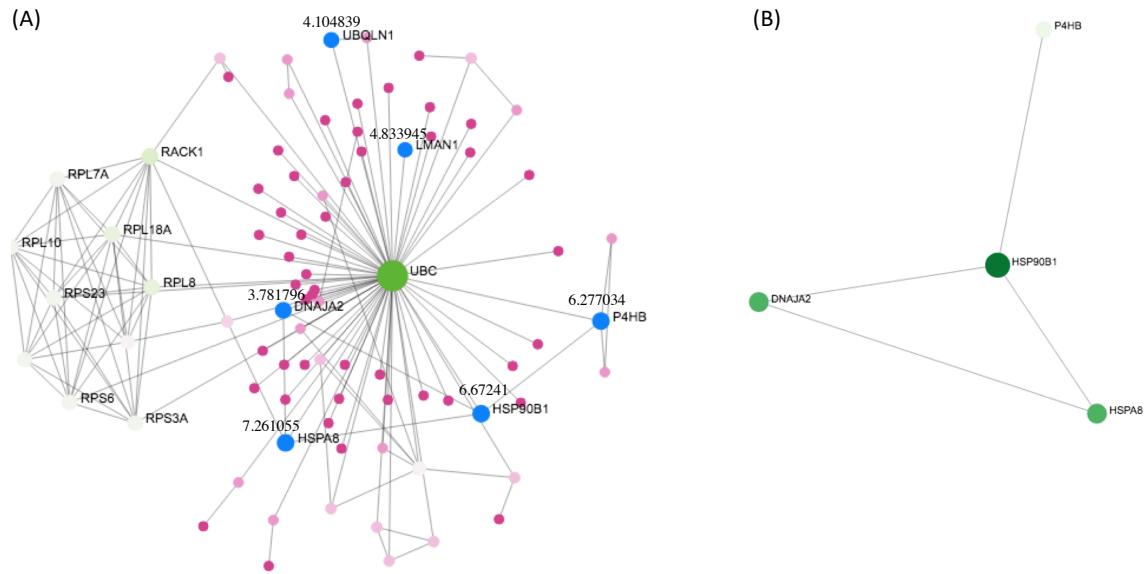
10399	RACK1	11	58.71	6.71587613
6142	RPL18A	10	56.87	6.71812216
6132	RPL8	10	56.87	6.14703757
6228	RPS23	9	38.72	5.46938802
6189	RPS3A	9	38.72	5.67086
6194	RPS6	9	38.72	5.43906883
6134	RPL10	9	0.78	7.22927072
6130	RPL7A	9	0.78	5.87863243
6176	RPLP1	8	0.5	4.95154703
60	ACTB	7	9.5	7.05993541
6170	RPL39	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 core of this project due to its close association with pancreatic cancer and other related diseases within all of the significantly (p value < 0.05) enriched pathways of DEGs is the endoplasmic reticulum protein processing pathway.

The endoplasmic reticulum (ER) is a cytoplasmic organelle in which excretory or membrane proteins are synthesized. Shortly, ER stress can be defined as a disparity between the ER's protein folding capacity and the protein load, resulting in the collection of cranking proteins (30,31). ER stress has been considered to be engaged in most 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 our findings, we speculate that the whole endoplasmic reticulum protein processing pathway might be unsettled in PDAC because of over-expression of ER-associated proteins Figure 5. There were 12 DEGs particularly engaged in this 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. Associated 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, transforming molecular chaperones with key roles in organizing and folding other proteins. The HSP90B1 protein is localized in the endoplasmic reticulum. Expression of HSP90B1 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 outcomes confirm the vital task of the pathway of the ER protein processing engaged in pancreatic cancer and related diseases treatment, offering new molecular therapeutic targets to improve fundamental drug agents.

Figure 5 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 is the key genes of subnetwork 2.

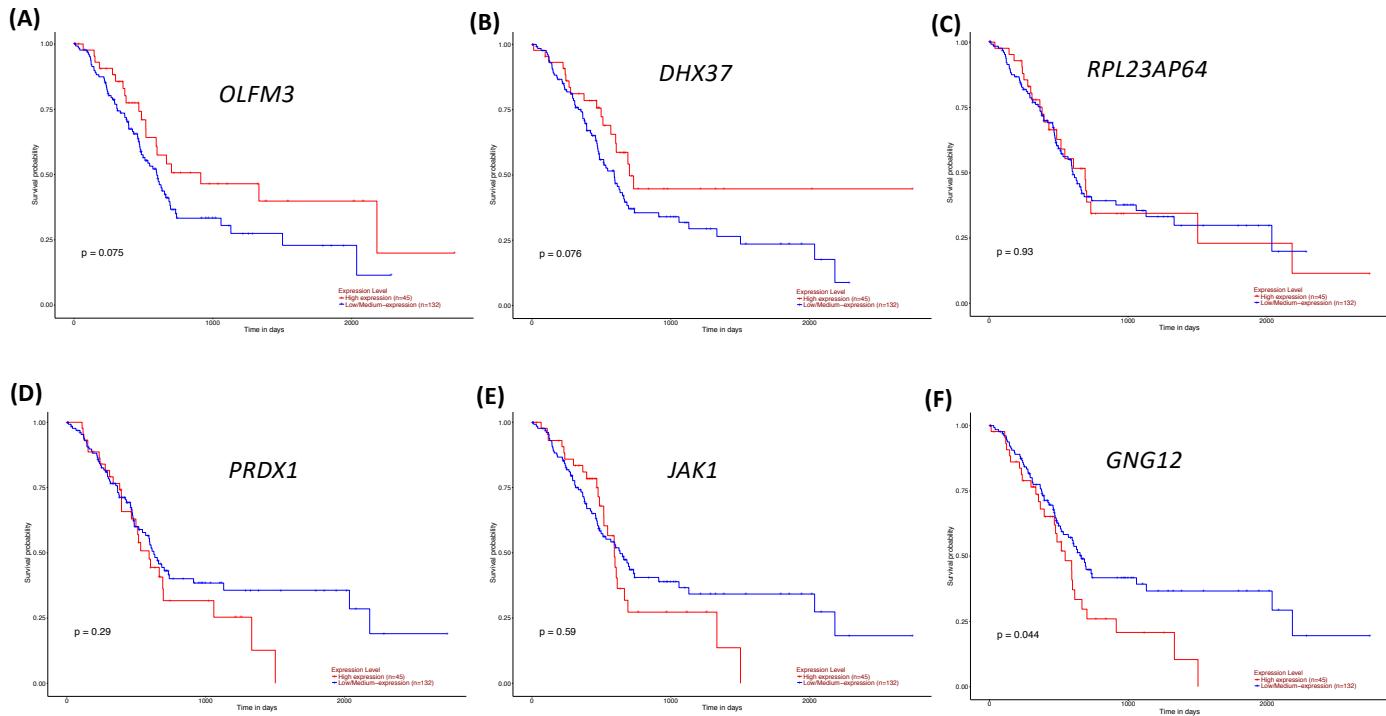


3.8 Survival Analysis

KM plotter was employed to predict the prognostic values of the 6 hub genes of down and up-regulated DEGs. Among the DEGs examined, our results showed 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 (17).

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 were only analyzed between tumor and normal tissues. 207 differentially expressed genes of up regulation, whereas we find 221 down-regulated differentially expressed genes were identified using R, and GO. And further KEGG pathway analyses were performed which showed locational and functional information of these differentially expressed genes. The outcomes of the GO enrichments shows that the majority of 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 indicated that mostly the upregulated DEGs were involved 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, hsa04972:pancreatic

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

Recently, it is identified an essential role for 'ribosome' pathway genes related to ribosome biogenesis in early pancreas development (34). Recent studies found that metabolic change is believed 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).

A 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 the development, 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 Endoplasmic reticulum (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, we analyzed the 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 essential to the molecular mechanisms underlying the development of PDAC and may therefore serve as potential therapeutic targets. Ubiquitin-C (UBC) was identified as one of the hub genes with the greatest degree of connectivity. A recent study revealed that UBC expression associates with increased patient survival in pancreatic ductal adenocarcinoma (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 studies have shown 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). In a most recent study, it was found that RACK1 was significantly up-regulated in human PDAC samples and cell lines (44). However, the exact role of RACK1 in human PDAC growth stays unidentified.

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 lowexpression 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 explore 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 pancreatic tumor and non-tumor datasets and 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.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin [Internet]. 2018 [cited 2020 Oct 22];68(1):7–30. Available from: <https://acsjournals.onlinelibrary.wiley.com/doi/abs/10.3322/caac.21442>
2. Gillen S, Schuster T, Büschenthalde CM zum, Friess H, Kleeff J. Preoperative/Neoadjuvant Therapy in Pancreatic Cancer: A Systematic Review and Meta-analysis of Response and Resection Percentages. PLOS Med [Internet]. 2010 Nis [cited 2020 Oct 22];7(4):e1000267. Available from: <https://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.1000267>
3. Tempero MA, Malafa MP, Behrman SW, Benson AB, Casper ES, Chiorean EG, et al. Pancreatic adenocarcinoma, version 2.2014. J Natl Compr Canc Netw. 2014;12(8):1083–93.
4. Lambert A, Schwarz L, Borbath I, Henry A, Van Laethem J-L, Malka D, et al. An update on treatment options for pancreatic adenocarcinoma. Ther Adv Med Oncol [Internet]. 2019 Jan [cited 2020 Oct 22];11:175883591987556. Available from: <http://journals.sagepub.com/doi/10.1177/1758835919875568>
5. Alldinger I, Dittert D, Peiper M, Fusco A, Chiappetta G, Staub E, et al. Gene expression analysis of pancreatic cell lines reveals genes overexpressed in pancreatic cancer. Pancreatology. 2005;5(4–5):370–9.
6. Jones S, Zhang X, Parsons DW, Lin JC-H, Leary RJ, Angenendt P, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. science. 2008;321(5897):1801–6.
7. Zhou W, Sokoll LJ, Bruzek DJ, Zhang L, Velculescu VE, Goldin SB, et al. Identifying markers for pancreatic cancer by gene expression analysis. Cancer Epidemiol Prev Biomark. 1998;7(2):109–12.

8. Grützmann R, Boriss H, Ammerpohl O, Lüttges J, Kalthoff H, Schackert HK, et al. Meta-analysis of microarray data on pancreatic cancer defines a set of commonly dysregulated genes. *Oncogene*. 2005;24(32):5079–88.
9. Grønborg M, Kristiansen TZ, Iwahori A, Chang R, Reddy R, Sato N, et al. Biomarker discovery from pancreatic cancer secretome using a differential proteomic approach. *Mol Cell Proteomics*. 2006;5(1):157–71.
10. Wang J, Yang S, He P, Schetter AJ, Gaedcke J, Ghadimi BM, et al. Endothelial Nitric Oxide Synthase Traffic Inducer (NOSTRIN) is a Negative Regulator of Disease Aggressiveness in Pancreatic Cancer. *Clin Cancer Res*. 2016 Dec 15;22(24):5992–6001.
11. Yang S, He P, Wang J, Schetter A, Tang W, Funamizu N, et al. A Novel MIF Signaling Pathway Drives the Malignant Character of Pancreatic Cancer by Targeting NR3C2. *Cancer Res*. 2016 Jul 1;76(13):3838–50.
12. Dineen SP, Roland CL, Greer R, Carbon JG, Toombs JE, Gupta P, et al. Smac mimetic increases chemotherapy response and improves survival in mice with pancreatic cancer. *Cancer Res*. 2010;70(7):2852–61.
13. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting Cancer Incidence and Deaths to 2030: The Unexpected Burden of Thyroid, Liver, and Pancreas Cancers in the United States. *Cancer Res* [Internet]. 2014 Jun 1 [cited 2020 Oct 28];74(11):2913–21. Available from: <http://cancerres.aacrjournals.org/cgi/doi/10.1158/0008-5472.CAN-14-0155>
14. Kamimura K, Yokoo T, Terai S. Gene Therapy for Pancreatic Diseases: Current Status. *Int J Mol Sci* [Internet]. 2018 Oct 31 [cited 2020 Oct 28];19(11):3415. Available from: <http://www.mdpi.com/1422-0067/19/11/3415>
15. Barrett T, Troup DB, Wilhite SE, Ledoux P, Rudnev D, Evangelista C, et al. NCBI GEO: mining tens of millions of expression profiles—database and tools update. *Nucleic Acids Res* [Internet]. 2007 Jan 1 [cited 2021 Jun 30];35(suppl_1):D760–5. Available from: <https://doi.org/10.1093/nar/gkl887>
16. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res*. 2002 Jan 1;30(1):207–10.
17. Lv K, Yang J, Sun J, Guan J. Identification of key candidate genes for pancreatic cancer by bioinformatics analysis. *Exp Ther Med* [Internet]. 2019 Jul 1 [cited 2020 Nov 12];18(1):451–8. Available from: <http://www.spandidos-publications.com/10.3892/etm.2019.7619/abstract>
18. Kwon M-S, Kim Y, Lee S, Namkung J, Yun T, Yi SG, et al. Integrative analysis of multi-omics data for identifying multi-markers for diagnosing pancreatic cancer.

BMC Genomics [Internet]. 2015 Aug 17 [cited 2021 Jun 28];16(9):S4. Available from: <https://doi.org/10.1186/1471-2164-16-S9-S4>

19. Davis S, Meltzer P. GEOquery: A bridge between the Gene Expression Omnibus (GEO) and BioConductor. *Bioinforma Oxf Engl*. 2007 Aug 1;23:1846–7.
20. Warnes GR, Bolker B, Bonebakker L, Gentleman R, Huber W, Liaw A, et al. gplots: Various R programming tools for plotting data. R Package Version. 2009;2(4):1.
21. Durinck S, Moreau Y, Kasprzyk A, Davis S, De Moor B, Brazma A, et al. BioMart and Bioconductor: a powerful link between biological databases and microarray data analysis. *Bioinformatics*. 2005;21(16):3439–40.
22. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Methodol*. 1995;57(1):289–300.
23. Dudoit S, Shaffer JP, Boldrick JC. Multiple hypothesis testing in microarray experiments. *Stat Sci*. 2003;71–103.
24. Tarca AL, Romero R, Draghici S. Analysis of microarray experiments of gene expression profiling. *Am J Obstet Gynecol*. 2006 Aug;195(2):373–88.
25. Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*. 2009;4(1):44.
26. Eden E, Navon R, Steinfeld I, Lipson D, Yakhini Z. GOrilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists. *BMC Bioinformatics*. 2009;10(1):1–7.
27. Zhou G, Soufan O, Ewald J, Hancock REW, Basu N, Xia J. NetworkAnalyst 3.0: a visual analytics platform for comprehensive gene expression profiling and meta-analysis. *Nucleic Acids Res*. 2019;47(W1):W234–41.
28. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, et al. STRING v10: protein–protein interaction networks, integrated over the tree of life. *Nucleic Acids Res*. 2015;43(D1):D447–52.
29. Chandrashekhar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BV, et al. UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. *Neoplasia*. 2017;19(8):649–58.
30. Kelly E, Greene CM, Carroll TP, McElvaney NG, O'Neill SJ. Selenoprotein S/SEPS1 modifies endoplasmic reticulum stress in Z variant alpha1-antitrypsin deficiency. *J Biol Chem*. 2009 Jun 19;284(25):16891–7.
31. Hidvegi T, Schmidt BZ, Hale P, Perlmutter DH. Accumulation of mutant alpha1-antitrypsin Z in the endoplasmic reticulum activates caspases-4 and -12,

- NFkappaB, and BAP31 but not the unfolded protein response. *J Biol Chem*. 2005 Nov 25;280(47):39002–15.
32. Forman MS, Lee VM-Y, Trojanowski JQ. “Unfolding” pathways in neurodegenerative disease. *Trends Neurosci*. 2003 Aug;26(8):407–10.
 33. Gow A, Sharma R. The unfolded protein response in protein aggregating diseases. *Neuromolecular Med*. 2003;4(1–2):73–94.
 34. Provost E, Weier CA, Leach SD. Multiple Ribosomal Proteins Are Expressed at High Levels in Developing Zebrafish Endoderm and Are Required for Normal Exocrine Pancreas Development. *Zebrafish [Internet]*. 2013 Jun [cited 2021 Jul 5];10(2):161–9. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3673614/>
 35. Grant TJ, Hua K, Singh A. Molecular Pathogenesis of Pancreatic Cancer. *Prog Mol Biol Transl Sci [Internet]*. 2016 [cited 2021 Jul 5];144:241–75. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6260831/>
 36. Wang W, Nag S, Zhang X, Wang M-H, Wang H, Zhou J, et al. Ribosomal Proteins and Human Diseases: Pathogenesis, Molecular Mechanisms, and Therapeutic Implications. *Med Res Rev [Internet]*. 2015 Mar [cited 2021 Jul 5];35(2):225–85. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4710177/>
 37. Burridge K. Focal adhesions: a personal perspective on a half century of progress. *FEBS J [Internet]*. 2017 [cited 2021 Jul 5];284(20):3355–61. Available from: <https://febs.onlinelibrary.wiley.com/doi/abs/10.1111/febs.14195>
 38. Jiang N, Dai Q, Su X, Fu J, Feng X, Peng J. Role of PI3K/AKT pathway in cancer: the framework of malignant behavior. *Mol Biol Rep [Internet]*. 2020 [cited 2020 Nov 2];47(6):4587–629. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7295848/>
 39. Zhu Y, Xu H, Chen H, Xie J, Shi M, Shen B, et al. Proteomic analysis of solid pseudopapillary tumor of the pancreas reveals dysfunction of the endoplasmic reticulum protein processing pathway. *Mol Cell Proteomics*. 2014;13(10):2593–603.
 40. Chu KY, Li H, Wada K, Johnson JD. Ubiquitin C-terminal hydrolase L1 is required for pancreatic beta cell survival and function in lipotoxic conditions. *Diabetologia [Internet]*. 2012 Jan 1 [cited 2021 Jul 5];55(1):128–40. Available from: <https://doi.org/10.1007/s00125-011-2323-1>
 41. Yang Y-H, Zhang Y-X, Gui Y, Liu J-B, Sun J-J, Fan H. Analysis of the autophagy gene expression profile of pancreatic cancer based on autophagy-related protein microtubule-associated protein 1A/1B-light chain 3. *World J Gastroenterol [Internet]*. 2019 May 7 [cited 2020 Nov 3];25(17):2086–98. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6506580/>

42. Adams DR, Ron D, Kiely PA. RACK1, A multifaceted scaffolding protein: Structure and function. *Cell Commun Signal.* 2011;9(1):1–24.
43. Wang L, Liu H-L, Li Y, Yuan P. Proteomic analysis of pancreatic intraepithelial neoplasia and pancreatic carcinoma in rat models. *World J Gastroenterol WJG.* 2011;17(11):1434.
44. Li X, Xiao Y, Fan S, Xiao M, Wang X, Chen X, et al. RACK1 overexpression associates with pancreatic ductal adenocarcinoma growth and poor prognosis. *Exp Mol Pathol.* 2016 Oct;101(2):176–86.