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## Identification of potential biomarkers in Pancreatic Adenocarcinoma of micro-array gene expression data

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Address

**Abstract:** We uncover molecular biomarkers using GSE16515 data set publicly reachable at NIH/NCBI Gene Expression Omnibus database. Using Biobase, GEOquery, gplots packages in R software 3.6. Based on gene expression analysis, we detect 278 differentially expressed genes (DEGs) of up regulation, whereas we find 77 down-regulated gene. The gene ontology of pathway enrichments and KEGG enrichment analyses of DEGs were studied. 120 KEGG pathways associated with pancreatic adenocarcinoma (PAAD) were identified, among which the PI3K-Akt signaling pathway was observed to be significant. Other hub genes discussed in this study, may be used as potential targets for PAAD and related diseases diagnosis and treatment. The following 21 hub genes were detected through NetworkAnalyst on the basis of protein-protein interaction network by the STRING tool: CDK1, CCNB1, CDC20, PPARG, MET, ISG15, LEF1, SFN, DMD, FN1, RUNX2, UBC, TOP2A, ECT2, WNT2, EFNA5, PAK3, PKM, ITGB4, NEK2, and ALB. The expression levels of PPARG and SFN were examined in TCGA database was significantly upregulated in pancreatic adenocarcinoma tumor cells compared with normal cells consistent with previous results. Additionally, the module analysis of protein-protein interactions revealed that ‘amoebiasis’, ‘focal adhesion’, ‘protein digestion and absorption’, and ‘ECM-receptor interaction’ had a close association with PAAD. In addition, PI3K/AKT signaling pathway in PAAD was observed to be significant. Other hub genes discussed in the study, might be utilized as promising targets for PAAD and related diseases diagnosis and drug therapies.

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

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## 1 Introduction

Pancreatic ductal adenocarcinoma (PAAD) is ranked as most mortal cancer due to the diagnosis at a distant stage which has a 5-year overall survival rate as 3% (Siegel et al., 2018). 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 (Gillen et al., 2010; Tempero et al., 2014). However, pancreatectomy still stays the most efficient treatment, specifically for initial phase pancreatic cancer (Lambert et al., 2019). 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 (W. Zhou et al., 1998). Recent studies suggest an extensive gene expression analysis of pancreatic cancer by reviewing gene expression data sets by comparing tumor and normal cells (Alldinger et al., 2005; Jones et al., 2008; W. Zhou et al., 1998).

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 (Grützmann et al., 2005). The protein expression alterations in the advancement and growth of pancreatic 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 (Grønborg et al., 2006).

Pei *et al.*, 2009, recently performed microarray experiments to study the differentially expressed levels of FKBP5 gene between the pancreatic tumor and normal samples (data obtainable at NCBI GEO database (Edgar et al., 2002), accession GSE16515)."

The DEGs of pancreatic tumor and normal samples were identified by comparing gene expression by fold change and t-test. Afterwards, the DEGs were filtered using online DAVID tool and the analysis of pathway enrichments (Sherman & Lempicki, 2009). By studying their hub nodes globally and between tumor and normal samples constructing PPI networks, the pupose of this project is to study the molecular and genetic mechanisms of pancreatic cancer 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 (Dineen et al., 2010)

Traditional therapeutic alternatives, particularly chemotherapy, are not efficient enough fighting PAAD, and notwithstanding advancements over the past 15 years, the survival rate has not increased and becoming the one of the most lethal cancer type (Rahib et al., 2014). Thus, constant efforts of the advancement of novel therapeutic alternatives is a need (Kamimura et al., 2018).

Much work has been done with microarray gene expression technology to reveal the central mechanism of pancreatic formation and progression and focus these methods for therapeutic approach. It still persists a request for more efficient treatments or methods that can improve curative responses to pancreatic cancer medication. In this project, we used microarray data sets of public transcriptome datasets of performed microarray experiments to identify the DEGs between the pancreatic tumor and normal samples.

Although experimental studies are needed to confirm our findings, our results will reveal potential biomarkers and bright therapeutic objectives for timely screening of pancreatic cancer. The present study also focused on the phosphoinositide-3/Akt (PI3K/Akt) signaling pathway and triggering receptor tyrosine kinases (RTKs) that plays a key task in regulating downstream responses, involving cell viability, growth cycle, proliferation, cell migration and vascularization, by phosphorylating a variety of intracellular proteins (Franke, 2008; Hemmings & Restuccia, 2012). The pathway exists in all cells of major eukaryotes and is extremely maintained (Manning & Cantley, 2007). The current and future impersonal studies of suppressants fighting PI3K/AKT pathway in cancers should be clarified further. Our goal is to characterize PI3K/AKT pathway in PAAD to the practice and put forward the updated propitious to the patients with PAAD for targeted therapies.

## 2 Materials and Methods

### 2.1. Preprocessing of the data set

The publicly accessible gene expression data set from pancreatic tumor and normal samples were pull out from the GEO database with GSE16515 (Pei et al., 2009). Genomic information ranging from gene sequences to protein structure predictions were obtained. As described by Pei et al., 2009, the data set contains a total of expression of 54,676 genes of in total 52 samples i.e., 36 tumor and 16 normal specimens.

Using the GEOquery package in Bioconductor following conventional procedures in R studio, the GSE16515 data set is studied (Durinck et al., 2009; Tarca et al., 2006). The other packages we used in R studio are as the following; Biobase, biomaRT and gplots packages (Davis & Meltzer, 2007; Durinck et al., 2005; Warnes et al., 2009). To estimate the adjusted *p value* and avoid Type I errors, we used Benjamini-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 gene ontology (GO) and pathway enrichment analysis, and false discovery rate (FDR) were computed (Benjamini & Hochberg, 1995; Dudoit et al., 2003; Hochberg & Tamhane, 1987).

### 2.2. Experimental data and analysis codes

Analysis were conducted in the R statistical environment. Sample codes and analysis of GSE16515 data can be found <https://github.com/mathbioGVN/GSE16515.HSC.microarray.project> repository. We compared samples into two groups provided that pancreatic tumor and normal cells. 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. The genes are filtered in accordance with both fold change and *p value* criterion. 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 (Tarca et al., 2006). We highlight statistical significance performing *t-test* by taking *p value* cutoff 0.01 and fold cut-off > 2 to identify down and up-regulated DEGs between each category understudy.

### 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. We used gplots package of R to create heatmaps of DEGs with heatmap.2 function. Clustering analysis of DEGs was performed to match the expression pattern of DEGs in tumor and normal cells.

## 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 gene names using Ensemble Biomart package in R. All of the DEGs were characterized by their biological processes, molecular functions, and cellular components of gene GO enrichment and DAVID which stands for Visualization and Integrated Discovery (Huang et al., 2007; Sherman & Lempicki, 2009). 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 (Eden et al., 2009; Supek et al., 2011). We then compared the results of DAVID with NetworkAnalyst enrichments performed with KEGG (G. Zhou et al., 2019).

## 2.5. Core genes filtered through the protein-protein interaction (PPI) network

NetworkAnalyst, publicly accessible on the web, provides analysis of PPI networks for single gene lists using STRING Interactome (Szkarczyk et al., 2015). To comprehensively decipher the regulatory mechanisms in pancreatic ad and related diseases, DEGs from pancreatic tumors and normal cells samples were analyzed to form a PPI network with previously reported GO classification and enrichment.

## 2.6. Core genes expression levels and survival analysis

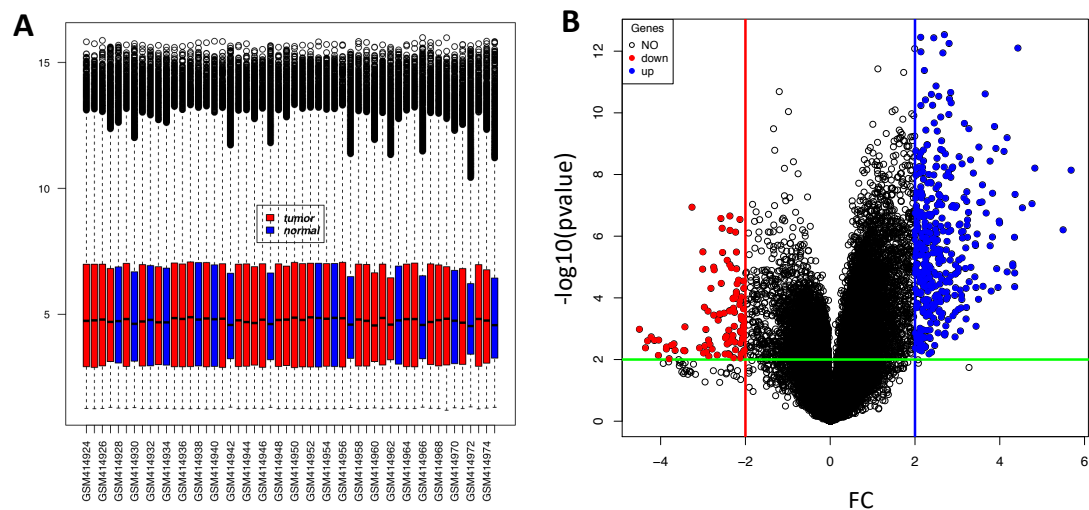
Based on the information of TCGA database, Ualcan (<http://ualcan.path.uab.edu/index.html>) (Chandrashekar et al., 2017) was utilized to conduct survival analysis. Kaplan-Meier survival analysis was carried out using 21 identified hub genes relying on the hub gene expression values in PAAD. In contrast to normal cells, gene expression levels presents important individual differences in tumor cells. Low expression level shows the transcripts per million (TPM) value is equal or below the upper quartile value whereas high expression level shows the TPM is above the upper quartile.

# 3 Results

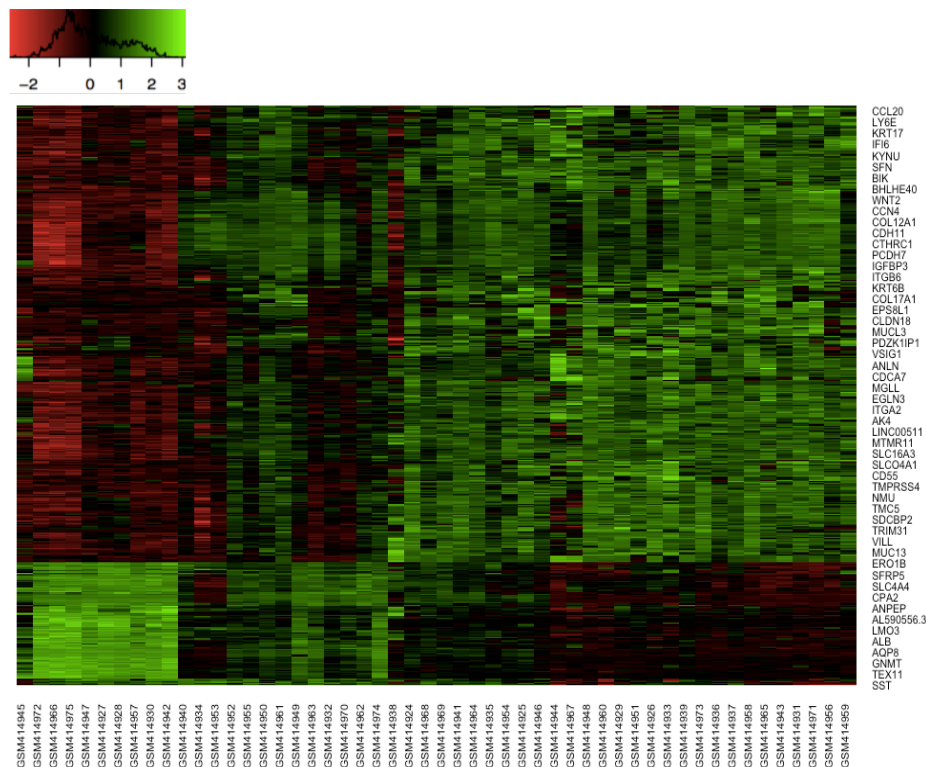
## 3.1. Experimental data analysis

With gene expression result of the GSE16515 data set, we detect differentially expressed genes (**DEGs**) in total 355 genes from **pancreatic tumor** and **normal cells** 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 fold change cut-off  $> 2$  and  $p < 0.01$ . Here, we detected 278 differentially expressed genes of up regulation, whereas we find 77 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 cells comparison. Black illustrates no change (NO), red illustrates down-regulated (Down), and blue illustrates up-regulated (Up) DEGs, FC, fold change.



**Figure 2** Heat map demonstrating DEGs in pancreatic tumor and normal cells aged samples. Each columns present samples, and rows present genes. Base-2 logarithmic values of the gene expression data are calculated. The progressive color changing from red to green represents the ranging from down to up-regulated DEGs.



### 3.2 Gene ontology and enrichment analysis

Table 2 shows the significant enrichment of DEGs using biological processes (BP) entailing collagen catabolic process, collagen fibril organization, extracellular matrix organization, and type I interferon signaling pathway. The significant enrichment of DEGs in cellular component (CC) contains extracellular exosome, extracellular space, and extracellular region. Finally, the significant enrichments GO terms in molecular function (MF) is revealed metalloendopeptidase activity, extracellular matrix structural constituent, and heparin binding.

KEGG signaling pathway study outcomes demonstrated in which these DEGs were considerably enriched in ECM-receptor interaction, focal adhesin, PI3K-Akt signaling pathway, and central carbon metabolism in cancer. Among these pathways, PI3K-Akt signaling pathway has vital influence on multiple cellular process which also has a role in cancer pathways almost in all cells (Jiang et al., 2020).

**Table 2.** Gene expression data set retrieved with top significant pathways GO enrichment analysis of DEGs in PAAD.

Category	Term	Count	p-value	Genes
BP	GO:0030574~collagen catabolic process	14	1.38E-10	MMP7, MMP1, COL11A1, COL12A1, KLK6, MMP12, COL1A1, MMP11, ADAMTS2, COL1A2, COL5A1, COL5A2, COL8A1, COL10A1
BP	GO:0030198~extracellular matrix organization	19	2.75E-08	ERO1A, ERO1B, POSTN, LAMB3, ITGB4, COL11A1, ITGA2, LAMA3, FN1, LAMC2, COL1A1, COMP, VCAN, COL1A2, COL5A1, COL5A2, COL8A1, COL10A1, ITGB6
BP	GO:0030199~collagen fibril organization	10	3.05E-08	GREM1, COL1A1, MMP11, ADAMTS2, COL1A2, COL5A1, COL11A1, COL12A1, COL5A2, LOXL2
BP	GO:0060337~type I interferon signaling pathway	10	2.64E-06	ISG20, IFI27, RSAD2, OAS1, OAS2, MX1, IFI6, ISG15, XAF1, OASL
CC	GO:0005615~extracellular space	77	6.52E-20	
CC	GO:0070062~extracellular exosome	105	4.85E-14	
CC	GO:0005576~extracellular region	72	6.01E-13	
CC	GO:0005578~proteinaceous extracellular matrix	22	1.67E-08	COL17A1, ECM1, POSTN, MMP7, MMP1, COL11A1, FN1, ADAMTS12, MMP12, COMP, MMP11, ADAMTS2, VCAN, COL1A2, COL5A1, COL5A2, MMP28, COL10A1, PI3, MUC4, WNT2, CTHRC1
MF	GO:0004222~metalloendopeptidase activity	12	6.95E-06	MMP12, MMP11, ADAMTS2, ADAM28, MMP7, FAP, MMP1, ADAM12, MMP28, ADAM9, ADAMTS12, KLK7
MF	GO:0005201~extracellular matrix structural constituent	9	2.87E-05	COL1A1, COMP, VCAN, COL1A2, COL5A1, COL11A1, COL5A2, MUC5B, MUC5AC
MF	GO:0008201~heparin binding	13	3.81E-05	POSTN, AOC1, MMP7, REG4, F11, FN1, LAMC2, CEL, THBS2, COMP, CXCL10, COL5A1, LIPH
KEGG	hsa04512:ECM-receptor interaction	15	3.15E-09	LAMB3, ITGB4, COL11A1, ITGA2, LAMA3, FN1, LAMC2, THBS2, COL1A1, COMP, COL1A2, COL5A1, COL5A2, SDC1, ITGB6
KEGG	hsa04510:Focal adhesin	17	3.15E-09	LAMB3, ITGB4, EGF, COL11A1, ITGA2, LAMA3, FN1, LAMC2, THBS2, COL1A1, COMP, COL1A2, COL5A1, COL5A2, ITGB6, PAK3, MET
KEGG	hsa04511:PI3K-Akt signaling pathway	17	02E-2	LAMB3, ITGB4, EGF, COL11A1, ITGA2, LAMA3, FN1, LAMC2, THBS2, EFNA5, COL1A1, COMP, COL1A2, COL5A1, COL5A2, ITGB6, MET
KEGG	hsa045230:Central carbon metabolism in cancer	6	0.1E-1	PKM, SLC2A1, SLC16A3, MET, HK2, PFKP

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



### 3.4. The PPI-network and KEGG pathway enrichment

Fig. 4 shows the PPI map between the set of input DEGs. The expressions of the nodes and their degree of connection were symbolized by yellow to red and fields, respectively in the visualized networks. The genes with the best three scores according to the *p value* is identified; these proteins also determines the functionality of the PPI network. Best scoring genes comprise with PPI network KEGG enrichment analysis represents involvement of ECM-receptor interaction (hsa04512), amoebiasis (hsa05146), protein digestion and absorption (hsa04974), focal adhesion (hsa04510), and PI3K-Akt signaling pathway (hsa04151). The other significantly enriched pathways associated with pancreatic tumor and normal cells DEGs are ECM-receptor interaction (hsa04512) and Central carbon metabolism in cancer (hsa0405230). Furthermore, the enriched KEGG pathways also involved the ‘PI3K-Akt signaling pathway’ due to playing a vital function in tissue development which is also associated with the cancerous cells.

**Figure 4** PPI network of DEGs was created and pictured using NetworkAnalyst. Progressive color change from yellow to the red represents relative gene expression value from down to up-regulated genes. CDK1 with the largest Betweenness Centrality (BC) was suggested to be central to the PPI network associated with pancreatic tumor and normal cells gene expression data. Moreover, CCNB1, CDC20, PPARG, MET, and ISG15 with the secondary highest degree and SFN with the secondary BC might be engaged in the progression pancreatic adenocarcinoma and other substance diseases.

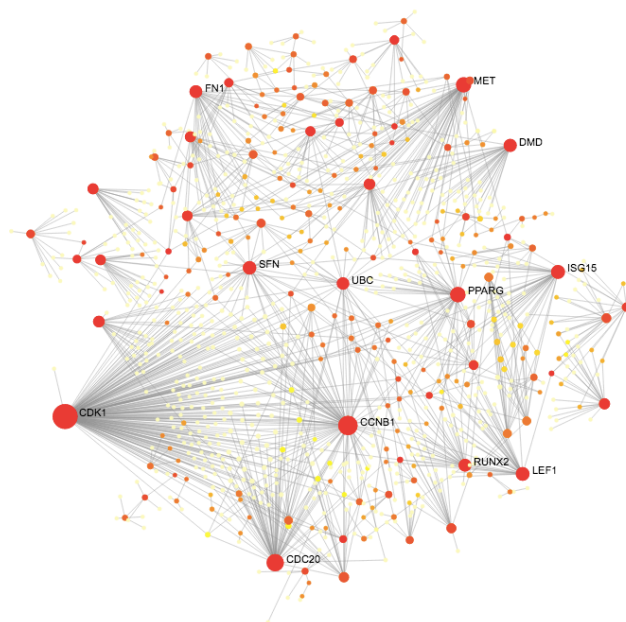


Fig.4 demonstrates PPI network of DEGs in pancreatic tumor and normal of all the DEGs . To conclude, PIP-network of pancreatic tumor and normal of all the DEGs (see Fig.4 and Table 3) in the expression data set. Hub genes in Fig.4 can be listed as CDK1, CCNB1, CDC20 , PPARG, MET, and ISG15. SFN gene is active in DNA repair, protein degradation, cell cycle regulation, endocytosis, control of cell signaling pathways, and kinase modification. UBC is a key gene that directly engages with other genes such as LC3, proposes that it might be a central component that heads to a bad prognosis of PAAD regulated by perineural invasion (Yang et al., 2019). ECT2 has a vital task in diseases comprising breast cancer and lung cancer. Related pathways of ECT2 are signaling pathways such as



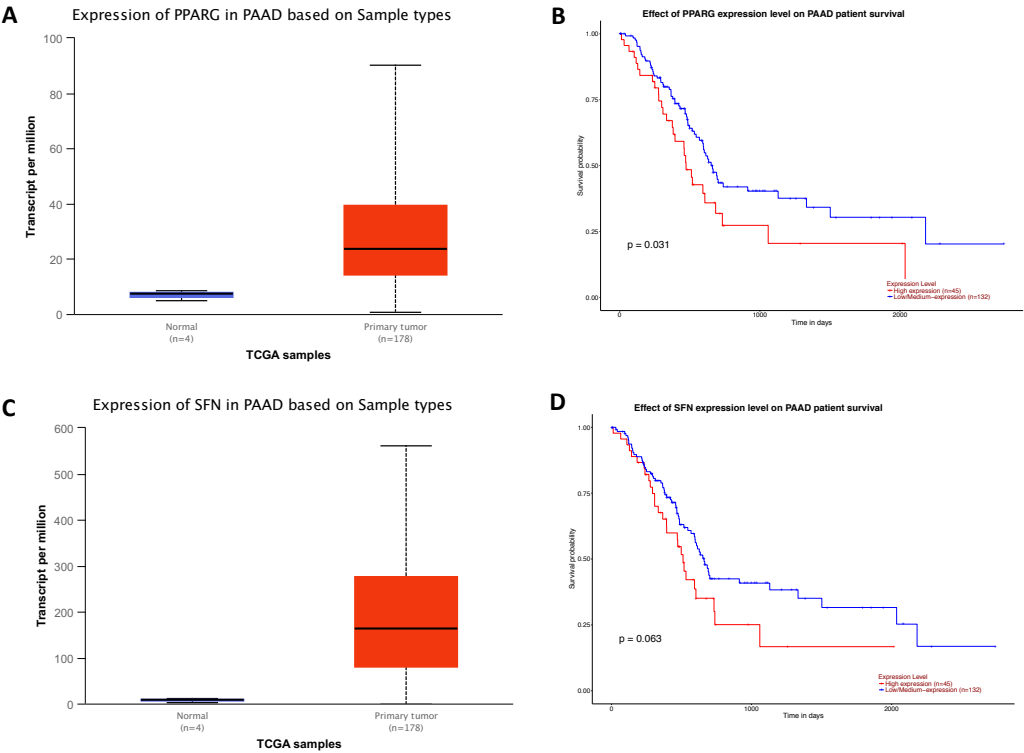
p75 NTR receptor-mediated signaling. GO annotations of molecular function related to this gene contain *GTPase activator activity* and *protein homodimerization activity*.

**Table 3** Top 15 genes of PIP network of DEGs in pancreatic tumor and normal cells gene expression data.

Gene ID	Genes	Node Degree	Betweenness centrality	Expression
983	CDK1	191	170861.12	6.95
891	CCNB1	91	42477.27	6.05
991	CDC20	66	30778.32	5.08
5468	PPARG	48	43020.9	6.84
4233	MET	47	76573.47	9.87
9636	ISG15	37	31351.4	8.86
51176	LEF1	36	52887.37	6.81
2810	SFN	34	28856.95	8.26
1756	DMD	32	37877.67	7.59
2335	FN1	30	36750.92	12.61
860	RUNX2	30	26338.94	7.37
7316	UBC	28	156179.54	8.81
7153	TOP2A	24	18346.88	6.74
1894	ECT2	23	16342.09	7.29

### 3.5. Pancreatic tumor gene expression and survival analyses through UALCAN portal

Notably, PPARG and SFN proteins are related to mortality and/or survival. Once the detection of hub proteins of PPI network was revealed, survival analysis for PAAD was carried out through UALCAN. *PPARG*; peroxisome proliferator-activated receptor gamma and SFN; Stratifin were shown to be significantly related to survival ( $P < 0.05$ ). Afterwards, the DEGs in PAAD were measured; PPARG and SFN were identified to be significantly differentially expressed. The levels of expression both *PPARG* were analyzed using TCGA database, and the outcomes match with those of the mentioned differential gene analysis; *PPARG* was significantly upregulated in pancreatic adenocarcinoma tumor cells compared with normal cells  $p = 1.62 \times 10^{-12}$ . In addition, SFN is also upregulated with in PAAD tumor and normal tissues with a  $p\text{-value} < 10^{-12}$ .



3.6. Validation of PPARG and SNF

45 tumor and 45 normal specimens of DEGs were studied. The expression level of PPARG and SFN was studied specifically. The outcomes of the analysis to validate the significance of PPARG and SFN are demonstrated in Table 4; it was noted that PPARG and SFN were expressed significantly upregulated in pancreatic adenocarcinoma cells in both GSE16515 and GSE28735.

**Table 4** Base-2 logarithmic scale of differential expression of most significant hub genes in pancreatic cancer cells in two different datasets.

Datasets	Genes	FC	P-value
GSE16515	PPARG	6.85	1.62E-12
	SFN	5.04	
GSE28735	PPARG	6.17	<1E-12
	SFN	4.8	

### 3.7. Role of the PI3K-Akt signaling pathway

The core of this project due to it is close association with cancer within all of the significantly ( $p$  value < 0.01) enriched pathways of DEGs is the PI3K-Akt signaling pathway. There were 17 DEGs particularly engaged in this pathway, containing LAMB3, ITGB4, EGF, COL11A1, ITGA2, LAMA3, FN1, LAMC2, THBS2, EFNA5, COL1A1, COMP, COL1A2, COL5A1, COL5A2, ITGB6, MET (Fig. 5 and Table 2 and 4). We have performed the primary DEGs associated with PI3K-Akt signaling pathway in Fig.6. We implemented PI3K-Akt signaling pathway genes to construct Fig.5A showing Subnetwork 1 and Fig.5B showing Subnetwork 2 protein-protein interaction networks.

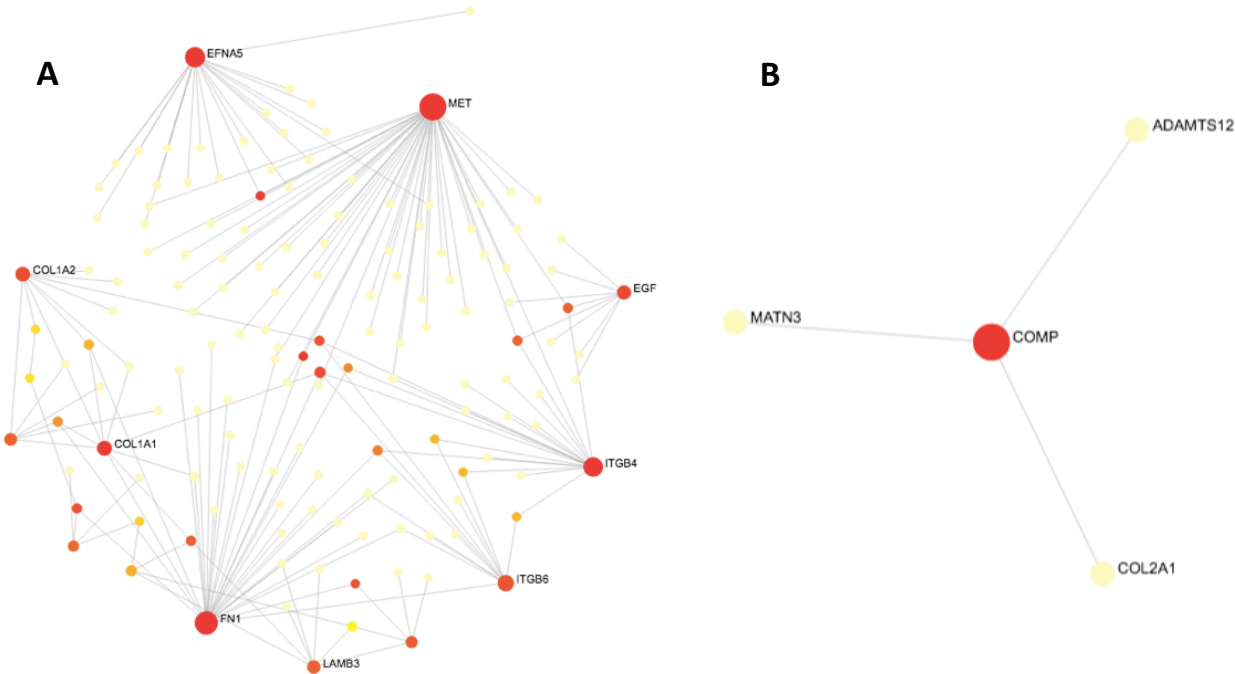
Associated genes with the DEGs of the data set enriched with the PI3K-Akt signaling pathway deciphered as new hub genes. In subnetwork 1 of PI3K-Akt pathway (Fig.5A), MET, FN1, and ITGB4 is the most significant genes in terms of BC. Moreover, collagen (COL) gene family also performs a key position which is demonstrated in the subnetwork of the PI3K-Akt signaling pathway. As shown in subnetwork 2, cartilage oligomeric matrix protein, COMP might be a gene that is associated with PI3K-Akt signaling pathway. We also found that the proteins associated with COMP gene in PI3K-Akt signaling pathway can be listed as ADAMTS12, MATN3, and COL2A1 in pancreatic tumor and normal cells gene expression data set. Even though the operational roles of other pathway members, such as ADAMTS12, stay unidentified downregulation or upregulation of particular ADAMTS proteins activated in serious human diseases, involving cancer. (Llamazares et al., 2007). These outcomes confirm the vital task of the PI3K-Akt signaling pathway engaged in pancreatic cancer and related diseases treatment, offering new molecular therapeutic targets to improve fundamental drug agents.

**Table 5** Top 10 most excessive KEGG pathway enrichment analysis of global DEGs in pancreatic tumor and normal cells micro-array gene expression data set.

Term	Count	P-value	Genes
hsa04512:ECM-receptor interaction	15	3.15E-09	LAMB3, ITGB4, COL11A1, ITGA2, LAMA3, FN1, LAMC2, THBS2, COL1A1, COMP, COL1A2, COL5A1, COL5A2, SDC1, ITGB6
hsa05146:Amoebiasis	13	2.27E-06	SERPINB3, CXCL8, SERPINB2, LAMB3, IL1R2, COL11A1, LAMA3, FN1, LAMC2, COL1A1, COL1A2, COL5A1, COL5A2
hsa04974:Protein digestion and absorption	12	2.28E-06	COL17A1, COL1A1, CPA2, COL1A2, CTRL, COL5A1, COL11A1, COL12A1, COL5A2, COL10A1, SLC16A10, KCNN4
hsa04510:Focal adhesion	17	7.13E-06	LAMB3, ITGB4, EGF, COL11A1, ITGA2, LAMA3, FN1, LAMC2, THBS2, COL1A1, COMP, COL1A2, COL5A1, COL5A2, ITGB6, PAK3, MET
hsa04151: PI3K-Akt signaling pathway	17	0.00271604	LAMB3, ITGB4, EGF, COL11A1, ITGA2, LAMA3, FN1, LAMC2, THBS2, EFNA5, COL1A1, COMP, COL1A2, COL5A1, COL5A2, ITGB6, MET
hsa05230:Central carbon metabolism in cancer	6	0.01154629	PKM, SLC2A1, SLC16A3, MET, HK2, PFKP
hsa00830:Retinol metabolism	6	0.01154629	SDR16C5, DHRS9, ADH1B, AOX1, UGT1A8, CYP2C18

hsa04610:Complement and coagulation cascades	6	0.01564074	C6, PLAUI, F11, PLAUIR, CD55, F5
hsa05144:Malaria	5	0.02015542	COMP, CXCL8, SDC1, THBS2, MET
hsa05200:Pathways in cancer	16	0.02027915	EGLN3, CXCL8, LAMB3, MMP1, EGF, ZBTB16, ITGA2, LAMA3, LEF1, SLC2A1, FN1, LAMC2, CKS2, PPARG, MET, WNT2

**Figure 5** Hub genes associated with PI3K-Akt signaling pathway. (A) Subnetwork 1 and (B) Subnetwork 2 protein-protein interaction networks.



4 Discussions

The prevalence of pancreatic adenocarcinoma and the related survival rates have demonstrated a decreasing in tendency in the past years (Siegel et al., 2018). One study showed that PAAD patients survive for only 4 months typically without therapies. Moreover, patients who undergo surgery and take required therapies the survival is not significantly increased (Wang et al., 2015). Thus, precise quick identification of PAAD and the advancement of powerful specific remedy is of fundamental significance.

A recent research detected hub genes in PAAD that were stated to be of diagnostic significance (Lv et al., 2019). In our study, the microarray GSE16515 of the gene expression database was conducted, holding gene expression of 36 tumor and 16 normal cells (Pei et al., 2009). Differentially expressed genes were only analyzed between tumor and normal cells. 278 upregulated and 77 downregulated differentially expressed genes were identified using R, and GO (Gene Ontology Consortium, 2004) and KEGG pathway analyses which showed locational and functional information of these differentially expressed genes. Upregulated DEGs were primarily positioned in the collagen trimers and extracellular exosome, and were engaged in cell adhesion, ECM organization and proteolysis, positive regulation of cell proliferation, collagen catabolic process and signal transduction. Contrary, downregulated DEGs were primarily enriched in proteolysis, digestion, and apoptotic process.

Finally, protein-protein interaction network construction show information about the core genes is used to identify function and disease associated with proteins. The highest interactive proteins CDK1, CCNB1, CDC20, PPARG, MET, LEF1, SFN, DMD, FN1, UBC, TOP2A, ECT2, WNT2, EFNA5, PAK3, PKM, ITGB4, NEK2, and ALB are predicted that are involved in several types of cancers like retinoblastoma, breast cancer, rhabdomyosarcoma, diabetes, childhood obesity, ovarian cancer, oral squamous cell carcinoma, acute lymphoblastic leukemia, colorectal and pancreatic cancer (Santiago et al., 2017; Sun et al., 2020; Tovar & Graveel, 2017; Tyagi et al., 2011). ISG15 pathway takes an important part in the tumorigenesis and treatment of digestive system cancers. (Zuo et al., 2016, p. 15). RUNX2 gene can be employed as molecular marker for diagnosis of initial stage of pancreatic cancer can also operate as promising a pro-oncogenic potential (Ozaki et al., 2018).

Finally, 21 core genes were detected using a PPI network and the prognostic measures of these hub genes for PAAD patients was studied through UACLAN. Utilizing data from TCGA that both *PPARG* and *SFN* were significantly upregulated in PAAD cells compared with control cells, as per the results of the differential gene analysis. Further, it was revealed that *SFN* and *PPARG* were considerably related with poorer survival. Thus, these two genes may be associated with the carcinogenesis and angiogenesis of PAAD.

After the enrichment analyses of KEGG and GO pathways, the functional enrichment of peroxisome proliferator activated receptor gamma (*PPARG*) was studied. *PPARG* is primarily involved in the modulation of fatty acid storage and glucose metabolism. *SFN* plays a function in kinase modification, endocytosis, DNA repair, protein degradation, cell cycle regulation, and modulation of various cell signaling pathways. Increased expression of *PPARG* might be the cause of the pathology of various diseases such as diabetes, obesity, atherosclerosis and pancreatic cancer. Therapies of inhibiting *SFN* downregulates Bcl-2 and XIAP expressions to stimulate apoptosis which directs to the downsizing in EMT, carcinogenic, angiogenic biomarkers with considerable suppression in tumor development in mice (Li et al., 2013).

This investigation further highlighted the PI3K-Akt signaling pathway involving differentially expressed genes in a broad various kinds of human cancer. AKT inhibitors have significant outlooks in the study of targeted cancer therapy since AKT is a vital transmitter of the PI3K-Akt signaling pathway (Jiang et al., 2020). The significant position of the PI3K-Akt signaling network in regulating cell proliferation, the cell cycle and apoptosis over the last 20 years was known (Hennessey et al., 2005; Rodon et al., 2013).

Furthermore, mutations employ in PI3K-Akt signaling pathway genes and many related pathway genes provide pancreatic ductal adenocarcinoma carcinogenesis. In addition to their standard roles, PI3K-Akt further rules metabolism characteristics of tumor cell and tumor microenvironment-intervened mechanisms of neoplasm development and survival (Murthy et al., 2018).

The presented project has certain limitations. In studying the expression level both PPARG and SFN only two datasets were analyzed, and more gene expression data should be investigated with a higher number of control samples is a need to verify the presented results. Secondly, additional studies is required for clinical lab confirmation of predicted proteins that are expressed in pancreatic tumor and normal cells data set and express at the developmental stage of pancreatic 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

- Alldinger, I., Dittert, D., Peiper, M., Fusco, A., Chiappetta, G., Staub, E., Löhr, M., Jesenofsky, R., Baretton, G., & Ockert, D. (2005). Gene expression analysis of pancreatic cell lines reveals genes overexpressed in pancreatic cancer. *Pancreatology*, 5(4–5), 370–379.
- Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, 57(1), 289–300.
- Chandrashekar, D. S., Bashel, B., Balasubramanya, S. A. H., Creighton, C. J., Ponce-Rodriguez, I., Chakravarthi, B. V., & Varambally, S. (2017). UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. *Neoplasia*, 19(8), 649–658.
- Davis, S., & Meltzer, P. (2007). GEOquery: A bridge between the Gene Expression Omnibus (GEO) and BioConductor. *Bioinformatics (Oxford, England)*, 23, 1846–1847.  
<https://doi.org/10.1093/bioinformatics/btm254>
- Dineen, S. P., Roland, C. L., Greer, R., Carbon, J. G., Toombs, J. E., Gupta, P., Bardeesy, N., Sun, H., Williams, N., & Minna, J. D. (2010). Smac mimetic increases chemotherapy response and improves survival in mice with pancreatic cancer. *Cancer Research*, 70(7), 2852–2861.
- Dudoit, S., Shaffer, J. P., & Boldrick, J. C. (2003). Multiple hypothesis testing in microarray experiments. *Statistical Science*, 71–103.
- Durinck, S., Moreau, Y., Kasprzyk, A., Davis, S., De Moor, B., Brazma, A., & Huber, W. (2005). BioMart and Bioconductor: A powerful link between biological databases and microarray data analysis. *Bioinformatics*, 21(16), 3439–3440.



- Durinck, S., Spellman, P. T., Birney, E., & Huber, W. (2009). Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt. *Nature Protocols*, 4(8), 1184.
- Eden, E., Navon, R., Steinfeld, I., Lipson, D., & Yakhini, Z. (2009). GOrilla: A tool for discovery and visualization of enriched GO terms in ranked gene lists. *BMC Bioinformatics*, 10(1), 1–7.
- Edgar, R., Domrachev, M., & Lash, A. E. (2002). Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Research*, 30(1), 207–210. <https://doi.org/10.1093/nar/30.1.207>
- Franke, T. F. (2008). PI3K/Akt: Getting it right matters. *Oncogene*, 27(50), 6473–6488. <https://doi.org/10.1038/onc.2008.313>
- Gene Ontology Consortium. (2004). The Gene Ontology (GO) database and informatics resource. *Nucleic Acids Research*, 32(suppl\_1), D258–D261.
- Gillen, S., Schuster, T., Büschenfelde, C. M. zum, Friess, H., & Kleeff, J. (2010). Preoperative/Neoadjuvant Therapy in Pancreatic Cancer: A Systematic Review and Meta-analysis of Response and Resection Percentages. *PLOS Medicine*, 7(4), e1000267. <https://doi.org/10.1371/journal.pmed.1000267>
- Grønborg, M., Kristiansen, T. Z., Iwahori, A., Chang, R., Reddy, R., Sato, N., Molina, H., Jensen, O. N., Hruban, R. H., & Goggins, M. G. (2006). Biomarker discovery from pancreatic cancer secretome using a differential proteomic approach. *Molecular & Cellular Proteomics*, 5(1), 157–171.

- Grützmann, R., Boriss, H., Ammerpohl, O., Lüttges, J., Kalthoff, H., Schackert, H. K., Klöppel, G., Saeger, H. D., & Pilarsky, C. (2005). Meta-analysis of microarray data on pancreatic cancer defines a set of commonly dysregulated genes. *Oncogene*, 24(32), 5079–5088.
- Hemmings, B. A., & Restuccia, D. F. (2012). PI3K-PKB/Akt Pathway. *Cold Spring Harbor Perspectives in Biology*, 4(9). <https://doi.org/10.1101/cshperspect.a011189>
- Hennessy, B. T., Smith, D. L., Ram, P. T., Lu, Y., & Mills, G. B. (2005). Exploiting the PI3K/AKT pathway for cancer drug discovery. *Nature Reviews Drug Discovery*, 4(12), 988–1004.
- Hochberg, Y., & Tamhane, A. C. (1987). *Multiple comparison procedures*. John Wiley & Sons, Inc.
- Hu, Y., Zeng, Q., Li, C., & Xie, Y. (2019). Expression profile and prognostic value of SFN in human ovarian cancer. *Bioscience Reports*, 39(5). <https://doi.org/10.1042/BSR20190100>
- Huang, D. W., Sherman, B. T., Tan, Q., Kir, J., Liu, D., Bryant, D., Guo, Y., Stephens, R., Baseler, M. W., & Lane, H. C. (2007). DAVID Bioinformatics Resources: Expanded annotation database and novel algorithms to better extract biology from large gene lists. *Nucleic Acids Research*, 35(suppl\_2), W169–W175.
- Jiang, N., Dai, Q., Su, X., Fu, J., Feng, X., & Peng, J. (2020). Role of PI3K/AKT pathway in cancer: The framework of malignant behavior. *Molecular Biology Reports*, 47(6), 4587–4629. <https://doi.org/10.1007/s11033-020-05435-1>
- Jones, S., Zhang, X., Parsons, D. W., Lin, J. C.-H., Leary, R. J., Angenendt, P., Mankoo, P., Carter, H., Kamiyama, H., & Jimeno, A. (2008). Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science*, 321(5897), 1801–1806.

- Kamimura, K., Yokoo, T., & Terai, S. (2018). Gene Therapy for Pancreatic Diseases: Current Status. *International Journal of Molecular Sciences*, 19(11), 3415.  
<https://doi.org/10.3390/ijms19113415>
- Lambert, A., Schwarz, L., Borbath, I., Henry, A., Van Laethem, J.-L., Malka, D., Ducreux, M., & Conroy, T. (2019). An update on treatment options for pancreatic adenocarcinoma. *Therapeutic Advances in Medical Oncology*, 11, 175883591987556.  
<https://doi.org/10.1177/1758835919875568>
- Li, S.-H., Fu, J., Watkins, D. N., Srivastava, R. K., & Shankar, S. (2013). Sulforaphane regulates self-renewal of pancreatic cancer stem cells through the modulation of Sonic hedgehog–GLI pathway. *Molecular and Cellular Biochemistry*, 373(1), 217–227.  
<https://doi.org/10.1007/s11010-012-1493-6>
- Llamazares, M., Obaya, A. J., Moncada-Pazos, A., Heljasvaara, R., Espada, J., López-Otín, C., & Cal, S. (2007). The ADAMTS12 metalloproteinase exhibits anti-tumorigenic properties through modulation of the Ras-dependent ERK signalling pathway. *Journal of Cell Science*, 120(20), 3544–3552. <https://doi.org/10.1242/jcs.005751>
- Lv, K., Yang, J., Sun, J., & Guan, J. (2019). Identification of key candidate genes for pancreatic cancer by bioinformatics analysis. *Experimental and Therapeutic Medicine*, 18(1), 451–458. <https://doi.org/10.3892/etm.2019.7619>
- Manning, B. D., & Cantley, L. C. (2007). AKT/PKB signaling: Navigating downstream. *Cell*, 129(7), 1261–1274. <https://doi.org/10.1016/j.cell.2007.06.009>
- Murthy, D., Attri, K. S., & Singh, P. K. (2018). Phosphoinositide 3-Kinase Signaling Pathway in Pancreatic Ductal Adenocarcinoma Progression, Pathogenesis, and Therapeutics. *Frontiers in Physiology*, 9. <https://doi.org/10.3389/fphys.2018.00335>

- Ozaki, T., Yu, M., Yin, D., Sun, D., Zhu, Y., Bu, Y., & Sang, M. (2018). Impact of RUNX2 on drug-resistant human pancreatic cancer cells with p53 mutations. *BMC Cancer*, 18(1), 309. <https://doi.org/10.1186/s12885-018-4217-9>
- Pei, H., Li, L., Fridley, B. L., Jenkins, G. D., Kalari, K. R., Lingle, W., Petersen, G., Lou, Z., & Wang, L. (2009). FKBP51 Affects Cancer Cell Response to Chemotherapy by Negatively Regulating Akt. *Cancer Cell*, 16(3), 259–266. <https://doi.org/10.1016/j.ccr.2009.07.016>
- Rahib, L., Smith, B. D., Aizenberg, R., Rosenzweig, A. B., Fleshman, J. M., & Matrisian, L. M. (2014). Projecting Cancer Incidence and Deaths to 2030: The Unexpected Burden of Thyroid, Liver, and Pancreas Cancers in the United States. *Cancer Research*, 74(11), 2913–2921. <https://doi.org/10.1158/0008-5472.CAN-14-0155>
- Rodon, J., Dienstmann, R., Serra, V., & Tabernero, J. (2013). Development of PI3K inhibitors: Lessons learned from early clinical trials. *Nature Reviews Clinical Oncology*, 10(3), 143–153.
- Santiago, L., Daniels, G., Wang, D., Deng, F.-M., & Lee, P. (2017). Wnt signaling pathway protein LEF1 in cancer, as a biomarker for prognosis and a target for treatment. *American Journal of Cancer Research*, 7(6), 1389–1406.
- Sherman, B. T., & Lempicki, R. A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols*, 4(1), 44.
- Siegel, R. L., Miller, K. D., & Jemal, A. (2018). Cancer statistics, 2018. *CA: A Cancer Journal for Clinicians*, 68(1), 7–30. <https://doi.org/10.3322/caac.21442>
- Sun, Y., Zhao, C., Ye, Y., Wang, Z., He, Y., Li, Y., & Mao, H. (2020). High expression of fibronectin 1 indicates poor prognosis in gastric cancer. *Oncology Letters*, 19(1), 93–102. <https://doi.org/10.3892/ol.2019.11088>

- Supek, F., Bošnjak, M., Škunca, N., & Šmuc, T. (2011). REVIGO summarizes and visualizes long lists of gene ontology terms. *PloS One*, 6(7), e21800.
- Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas, J., Simonovic, M., Roth, A., Santos, A., & Tsafou, K. P. (2015). STRING v10: Protein–protein interaction networks, integrated over the tree of life. *Nucleic Acids Research*, 43(D1), D447–D452.
- Tarca, A. L., Romero, R., & Draghici, S. (2006). Analysis of microarray experiments of gene expression profiling. *American Journal of Obstetrics and Gynecology*, 195(2), 373–388. <https://doi.org/10.1016/j.ajog.2006.07.001>
- Tempero, M. A., Malafa, M. P., Behrman, S. W., Benson, A. B., Casper, E. S., Chiorean, E. G., Chung, V., Cohen, S. J., Czito, B., & Engebretson, A. (2014). Pancreatic adenocarcinoma, version 2.2014. *Journal of the National Comprehensive Cancer Network*, 12(8), 1083–1093.
- Tovar, E. A., & Graveel, C. R. (2017). MET in human cancer: Germline and somatic mutations. *Annals of Translational Medicine*, 5(10). <https://doi.org/10.21037/atm.2017.03.64>
- Tyagi, S., Gupta, P., Saini, A. S., Kaushal, C., & Sharma, S. (2011). The peroxisome proliferator-activated receptor: A family of nuclear receptors role in various diseases. *Journal of Advanced Pharmaceutical Technology & Research*, 2(4), 236–240. <https://doi.org/10.4103/2231-4040.90879>
- Wang, X., Wang, L., Mo, Q., Dong, Y., Wang, G., & Jia, A. (n.d.). *Changes of Th17/Treg cell and related cytokines in pancreatic cancer patients*. 7.

- Warnes, G. R., Bolker, B., Bonebakker, L., Gentleman, R., Huber, W., Liaw, A., Lumley, T., Maechler, M., Magnusson, A., & Moeller, S. (2009). gplots: Various R programming tools for plotting data. *R Package Version*, 2(4), 1.
- Xia, J., Gill, E. E., & Hancock, R. E. W. (2015). NetworkAnalyst for statistical, visual and network-based meta-analysis of gene expression data. *Nature Protocols*, 10(6), 823–844.
- Yang, Y.-H., Zhang, Y.-X., Gui, Y., Liu, J.-B., Sun, J.-J., & Fan, H. (2019). Analysis of the autophagy gene expression profile of pancreatic cancer based on autophagy-related protein microtubule-associated protein 1A/1B-light chain 3. *World Journal of Gastroenterology*, 25(17), 2086–2098. <https://doi.org/10.3748/wjg.v25.i17.2086>
- Zhou, G., Soufan, O., Ewald, J., Hancock, R. E. W., Basu, N., & Xia, J. (2019). NetworkAnalyst 3.0: A visual analytics platform for comprehensive gene expression profiling and meta-analysis. *Nucleic Acids Research*, 47(W1), W234–W241.
- Zhou, W., Sokoll, L. J., Bruzek, D. J., Zhang, L., Velculescu, V. E., Goldin, S. B., Hruban, R. H., Kern, S. E., Hamilton, S. R., & Chan, D. W. (1998). Identifying markers for pancreatic cancer by gene expression analysis. *Cancer Epidemiology and Prevention Biomarkers*, 7(2), 109–112.
- Zuo, C., Sheng, X., Ma, M., Xia, M., & Ouyang, L. (2016). ISG15 in the tumorigenesis and treatment of cancer: An emerging role in malignancies of the digestive system. *Oncotarget*, 7(45), 74393–74409. <https://doi.org/10.18632/oncotarget.11911>