Integrated analysis of mRNA and miRNA expression profiles in pancreatic ductal adenocarcinoma

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Abstract. In the present study, to investigate the potential molecular mechanism of pancreatic ductal adenocarcinoma (PDAC), mRNA and miRNA expression profiles were integrated for systematic analysis. Results showed that a total of 76 common differentially expressed genes (DEGs) were identified from 2 mRNA expression profiles that contained 39 tumor and 15 normal samples. Notably, the tumor and normal samples were able to be clearly classified into 4 groups based on the DEGs. mRNA-miRNA regulation network analysis indicated that 22 out of the 76 DEGs including *MUC4*, *RRM2* and *CCL2* are regulated by 5 reported miRNAs. Survival analysis using SurvExpress database demonstrated that the common DEGs were able to significantly differentiate low- and high-risk PDAC groups in 4 datasets. In summary, various biological processes are probably involved in the development and progression of PDAC. Firstly, activation of *MUC4* induces nuclear translocation of β-catenin and promotes the process of angiogenesis that provides necessary nutrition or oxygen for cancer cells. Then, *RRM2* induces the invasiveness of PDAC via *NF-κB*. Finally, the formation of an immunosuppressive tumor microenvironment by recruiting regulatory T cells with high expression of *CCL2* further promotes cancer cell proliferation and vascularization. Identification of valuable biological processes and genes can be helpful for the understanding of the molecular mechanism of PDAC.

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Introduction

Pancreatic cancer is the fourth most common cause of global cancer-related deaths (1) There were ~44,000 newly diagnosed cases of pancreatic cancer and more than 37,000 related deaths in 2012 in the US (2). In addition, the average 5-year survival rate is ~7.7% based on the data from 2006 to 2012 (3). Pancreatic ductal adenocarcinoma (PDAC), the most common histological subtype, comprises 90% of all pancreatic cancer cases (4). PDAC always displays local invasion and distant metastasis during early stages leading to poor prognosis with an overall 5-year survival rate of only 5% (5). World Health Organization (WHO) and Surveillance, Epidemiology and End Results (SEER) mortality data indicate that the occurrence of PDAC increases with age (~71 years) (6). In addition, researchers have shown that various risk factors can contribute to the development of PDAC. In Italy, PDAC risk was found to be 4.3-fold higher in heavy smokers (>20 cigarettes/day) compared with never smokers (7). In addition, alcohol intake was found to be associated with PDAC mortality based on data from the Cancer Prevention Study II. The results show evidence that alcohol consumption promotes PDAC mortality (8).

To explore the molecular mechanism of PDAC, numerous studies have been carried out using advanced microarray or next-generation sequencing technology. Previous studies based on microarrays have identified several genes that play an important role in PDAC. *GNAI2*, G protein subunit αi2, was found to be significantly upregulated in PDAC, and can mediate the functions of dopamine receptor D2 (*DRD2*) on cAMP signaling. Knockdown or inhibition of *DRD2* was found to reduce the proliferation of PDAC cells (9). Teodorczyk *at al* revealed that *CD95* is associated with stemness and epithelial-mesenchymal transition (EMT) in PDAC based on an *in silico* analysis of 36 RNA profiles (10). An *in vitro* experiment demonstrated that PDAC growth and metastasis can be significantly reduced by pharmacological inhibition of *CD95* activity, and *Sck* is necessary for the *CD95* induction of cell cycle progression (10). In addition, expression levels of microRNAs and lncRNAs were also explored in PDAC, and several markers have been identified including miR-10b, miR-155, miR-106b (11) and lncRNA *AFAP1-AS1* (12). Recently, whole-exome sequencing of 109 micro-dissected PDAC samples identified multiple novel mutated genes in PDAC such as *RBM10*, *KRAS*, *BRAF* and high-frequency

Table I. The identified 76 common DEGs in GSE32676 and GSE71989.

Gene	Fold-change		Gene	Fold-change	
	GSE32676	GSE71989		GSE32676	GSE71989
CCL2	-3.71	2.77	*LAMA3*	3.75	2.80
TMC5	3.33	2.93	*NR4A2*	-2.28	2.50
GJB2	4.54	2.58	*HOXB3*	2.08	2.09
DPCR1	4.23	2.29	*EFNA5*	2.12	2.16
MMP11	2.88	2.92	*ANLN*	2.39	2.08
CCL8	-3.23	2.12	*CTSE*	5.12	3.49
LCN2	4.59	2.18	*ANO1*	2.83	2.80
ZWINT	2.30	2.12	*MTUS2*	-2.32	-2.39
IFI27	2.10	3.37	*TSPAN1*	4.41	2.18
MMP19	-3.00	2.39	*C15orf48*	3.77	2.57
NQO1	3.09	3.41	*MMP28*	3.06	2.26
SLC6A14	5.88	3.34	*C19orf33*	5.67	2.99
ADAMTS12	2.69	2.41	*LAMC2*	2.25	2.37
RRM2	2.40	2.38	*VILL*	2.98	2.10
SFTA2	4.14	2.27	*SERPINB5*	5.62	2.29
PTGDS	-2.79	2.17	*CAMK2N1*	2.08	2.47
LAMB3	4.87	2.48	*ST6GALNAC1*	3.75	2.61
GPRC5A	3.14	3.46	*ETV1*	2.18	2.38
PHLDA2	3.04	3.40	*DCBLD1*	2.26	2.40
PPP1R1A	-2.42	-2.03	*CST1*	4.90	2.25
OAS1	2.51	2.10	*GCNT3*	3.93	2.38
ECT2	2.40	3.04	*SOCS3*	-2.43	3.25
SDR16C5	4.78	3.21	*MUC4*	4.30	2.44
LOC100505984	4.96	3.34	*SDC1*	2.68	2.36
MALL	2.13	2.34	*EPPK1*	3.54	2.31
THBS1	-3.11	2.60	*AGR2*	3.96	2.45
CEACAM1	3.47	2.08	*AGR3*	3.76	3.11
CLDN23	3.21	2.21	*SFN*	2.64	3.75
KLF5	3.09	2.09	*CDK1*	2.60	2.21
CEACAM5	6.81	3.96	*OGN*	-3.20	2.42
ITGA2	3.33	2.65	*AOC1*	3.76	2.44
CEACAM6	5.85	4.22	*EMP1*	-2.63	3.38
KRT17	4.01	3.00	*S100P*	6.75	4.78
S100A6	3.11	2.92	*AHNAK2*	3.76	2.55
FOSB	-3.97	3.40	*MSLN*	6.44	3.48
TFF1	5.06	2.92	*CAPN8*	5.28	2.99
TOP2A	2.76	3.21	*KRT19*	6.22	3.93
LY6E	2.16	2.37	*CH25H*	-2.42	2.06

DEGs, differentially expressed genes.

alterations in Wnt signaling, chromatin remodelling and cell cycle pathways (13).

Research has been carried out to explore the molecular mechanisms of PDAC based on microarray expression profiles or next-generation sequencing. However, studies with the integration of mRNA and miRNA expression profiles have not been widely applied in PDAC. In recent years, more and more microarray expression datasets have been submitted to the Gene Expression Omnibus (GEO) database, and re-analysis of the deposited datasets with various bioinformatics algorithms can be helpful (14). In the present study, we firstly identified the common differentially expressed genes (DEGs) in PDAC based on 2 mRNA expression profiles from 2 independent laboratories. Then functional annotation of the common DEGs based on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway databases was carried out. Furthermore, an interaction network between the identified DEGs and documented miRNAs was constructed. Finally, the identified DEGs were virtually validated using SurvExpress online database.

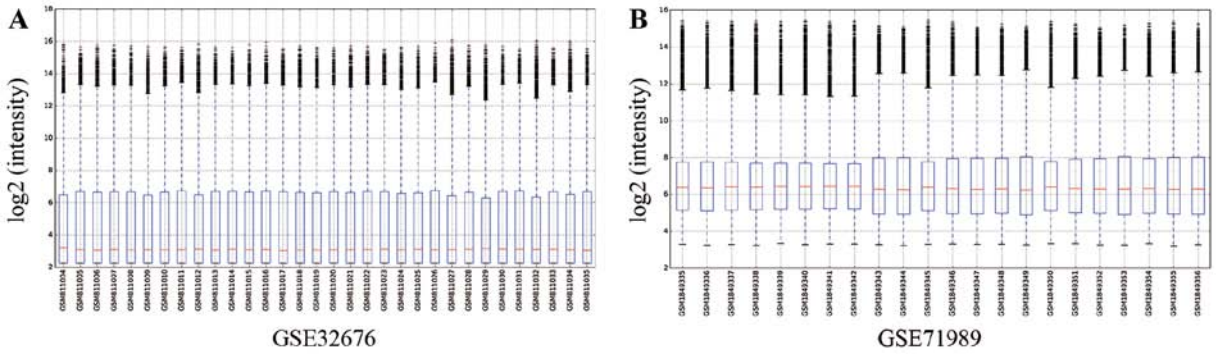

Figure 1. Boxplots for the gene expression of each sample in (A) GSE32676 and (B) GSE71989.

Materials and methods

Acquisition of mRNA and miRNA expression profiles. In the present study, publicly available datasets from Expression Omnibus Database (GEO) (http://www.ncbi.nlm.nih.gov/geo/) were used. Firstly, we carefully searched the GEO database, and downloaded 2 mRNA expression profiles. GSE71989 submitted by Thomas Schmittgen in 2015 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE71989) consists of 14 PDAC and 8 normal pancreas tissues. The other dataset GSE32676 submitted by Tran in 2011 (15) consists of 25 PDAC and 7 normal pancreas tissues. Based on the instruction from manufacturer, RNAs were extracted and hybridized to Affymetrix Human Genome U133 Plus 2.0 array. Detailed sample information and experiment designs were documented in the previous studies.

Differentially expressed gene screening. Data analysis was carried out using in-house R script and publicly available annotation database. In brief, mRNA expression values were firstly subject to background correction, normalization and $\log_2$ transformation using GeneChip Robust Multi-array Analysis (GC-RMA) algorithm (16). Furthermore, uninformative control probe sets were filtered out. In addition, the average expression value was calculated for the genes with multiple probes. Finally, DEGs were screened using Linear Models for Microarray Data (Limma) package (17) within the bioconductor. The criteria were set to adjust p-value ≤0.05 and $|\log_2$ fold-change (FC)$| \geq 2$. In addition, the common DEGs between the 2 datasets were identified based on a Venn diagram. The common DEGs were used to construct a heat map using Heatmap.2 method within ggplot package (18).

GO and KEGG pathway annotation. The functions of the identified DEGs were further annotated using GO and KEGG pathway databases using the online tools of Database for Annotation, Visualization and Integrated Discovery (DAVID) (19). The GO term consist of biological process (BP), cellular component (CC) and molecular function (MF). The criterion was set to $p<0.05$.

mRNA-miRNA interaction network. Numerous studies show that miRNAs play an important role in the regulation of carcinogenesis, malignant transformation and metastatic processes by preventing mRNA expression or via other processes (20). Cote *et al* (11) showed that 5 miRNAs, miR-10b-5p, miR-155-5p, miR-106b-5p, miR-30c-5p and miR-212-3p, have excellent performance to distinguish PDAC from normal samples. In addition, the sensitivity and specificity were 96 and 100%, respectively, in the training and validation cohorts. In the present study, we constructed the mRNA-miRNA interaction network based on the common DEGs and the 5 miRNAs. In brief, target genes of the 5 miRNAs were predicted based on the microCosm, mirTarbase and TargetScan databases. Then, the intersection between the common DEGs and the target genes were selected. Finally, the interaction network was constructed using CyTargetLinker (21) plugin in Cytoscape (22).

Virtual validation of the common DEGs. Clinical outcomes of the DEGs are critical for the diagnosis or treatment of PDAC. In the present study, virtual validation of the DEGs was carried out using SurvExpress online tool (23). This tool is based on a cancer-wide gene expression database with clinical outcomes. Four datasets were used for the virtual validation including GSE21501, GSE28735, TCGA PDAC and ICGC PDAC. Detailed information for the datasets can be found in previous studies. Parameter setting were carefully selected according to the developer's instructions.

Results

DEGs in PDAC. After background correction and normalization, the gene expression median values for different samples in the 2 datasets were almost at the same level (Fig. 1). Then, the datasets were subjected to DEG analysis. Results showed that a total of 364 and 816 DEGs were screened out for GSE32676 and GSE71989, respectively. For GSE32676, 292 genes (80.2%) were upregulated and 72 genes (19.8%) were downregulated. For GSE71989, 666 genes (81.6%) were upregulated and 150 genes (18.4%) were downregulated. Among those DEGs, 76 genes were differentially expressed in both GSE32676 and GSE71989 (Fig. 2). Fold-changes for the common DEGs are listed in Table I.

Furthermore, all samples from the 2 datasets were subjected to hierarchical clustering analysis based on the common DEGs. As shown in Fig. 3, tumor (red) and normal (blue) samples were abe to be clearly classified into different subgroups. Due to tumor heterogeneity and expression value variation, a few normal and tumor samples were erroneously classified.

Figure 2. Venn diagram of the differentially expressed genes (DEGs) in GSE32676 and GSE71989.

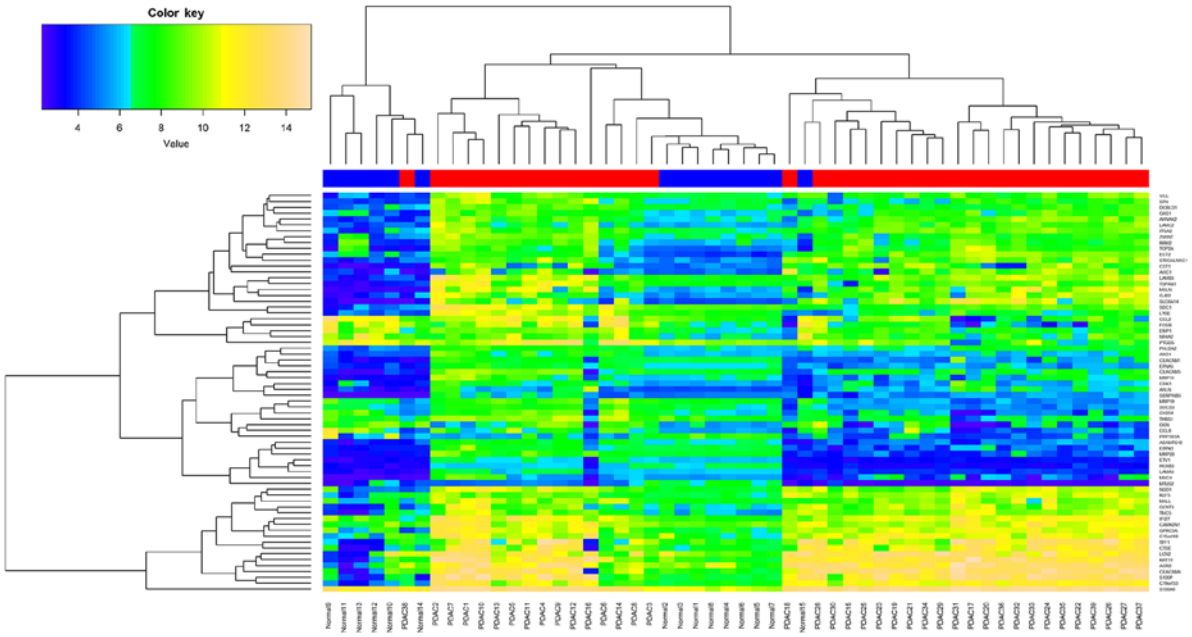

Figure 3. Heat map showing the differential expression pattern of 76 genes out of 1,104 differentially expressed genes (DEGs) in all samples. The x-axis represents samples and the bar on the top indicates the tumor samples (red) and the normal samples (blue). The y-axis represents the 76 genes.

Table II. KEGG pathway enrichment result for the common DEGs.

Term	Genes	P-value
hsa04512:ECM-receptor interaction	*LAMB3*, *SDC1*, *LAMA3*, *ITGA2*, *LAMC2*, *THBS1*	1.39E-05
hsa04115:p53 signaling pathway	*CDK1*, *SERPINB5*, *RRM2*, *SFN*, *THBS1*	1.21E-04
hsa05222:Small cell lung cancer	*LAMB3*, *LAMA3*, *ITGA2*, *LAMC2*	0.0040471
hsa04510:Focal adhesion	*LAMB3*, *LAMA3*, *ITGA2*, *LAMC2*, *THBS1*	0.0069739

KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes.

GO and KEGG pathway annotation. In order to explore the biological functions of the common DEGs, functional annotation of the common DEGs was carried out using DAVID online tool based on GO and KEGG pathway databases. Results showed that the common DEGs can be significantly enriched into 4 KEGG pathways (Table II). Six genes participated in the ECM-receptor interaction pathway (p=0.000013), 5 genes were involved in the p53 signaling pathway (p=0.00012), 4 genes mapped to small cell lung cancer (p=0.0040) and 5 genes play a role in the pathway of focal adhesion (p=0.0069). In addition, the common DEGs were mainly related to the biological process of epidermis development (p=0.0001), ectoderm development (p=0.0002) and cell adhesion (p=0.0008) (Table III). In addition, the top 5 cellular components include extracellular matrix (p=0.0), extracellular region (p=0.0), extracellular region part (p=0.0), proteinaceous extracellular matrix (p=0.0002) and anchored to membrane (p=0.023) (Table III). In addition, 5 signifi-

Table III. Top 5 GO terms for the common DEGs.

ID	GO term	P-values	Genes
BP			
GO:0008544	Epidermis development	0.0001	*LAMB3*, *LAMA3*, *KRT17*, *AHNAK2*, *LAMC2*, *SFN*, *EMP1*
GO:0007398	Ectoderm development	0.0002	*LAMB3*, *LAMA3*, *KRT17*, *AHNAK2*, *LAMC2*, *SFN*, *EMP1*
GO:0007155	Cell adhesion	0.0008	*DCBLD1*, *LAMB3*, *LAMA3*, *CCL2*, *MSLN*, *ITGA2*, *LAMC2*, *THBS1*, *CEACAM1*, *CLDN23*, *MUC4*
GO:0022610	Biological adhesion	0.0008	*DCBLD1*, *LAMB3*, *LAMA3*, *CCL2*, *MSLN*, *ITGA2*, *LAMC2*, *THBS1*, *CEACAM1*, *CLDN23*, *MUC4*
GO:0048545	Response to steroid hormone stimulus	0.0015	*KRT19*, *SDC1*, *CCL2*, *SOCS3*, *TFF1*, *THBS1*
CC			
GO:0031012	Extracellular matrix	0.0000	*OGN*, *LAMB3*, *LAMA3*, *MMP19*, *LAMC2*, *MMP28*, *ADAMTS12*, *THBS1*, *MMP11*, *MUC4*
GO:0005576	Extracellular region	0.0000	*CCL2*, *MMP19*, *CCL8*, *CST1*, *MMP28*, *SFN*, *MUC4*, *MMP11*, *LCN2*, *OGN*, *LAMB3*, *LAMA3*, *PTGDS*, *SERPINB5*, *MSLN*, *SFTA2*, *LAMC2*, *EFNA5*, *ADAMTS12*, *TFF1*, *AGR3*, *THBS1*, *AGR2*, *CEACAM1*
GO:0044421	Extracellular region part	0.0000	*CCL2*, *MMP19*, *CCL8*, *MMP28*, *SFN*, *MMP11*, *MUC4*, *OGN* *LAMB3*, *LAMA3*, *SERPINB5*, *LAMC2*, *EFNA5, TFF1, ADAMTS12*, *THBS1*
GO:0005578	Proteinaceous extracellular matrix	0.0002	*OGN*, *LAMB3*, *LAMA3*, *MMP19*, *LAMC2*, *MMP28*, *ADAMTS12*, *MMP11*, *MUC4*
GO:0031225	Anchored to membrane	0.0231	*LY6E*, *MSLN*, *CEACAM6*, *CEACAM5*, *EFNA5*
MF			
GO:0005198	Structural molecule activity	0.0035	*KRT19*, *LAMB3*, *LAMA3*, *EPPK1*, *KRT17*, *THBS1*, *VILL, CLDN23*, *MUC4*
GO:0004857	Enzyme inhibitor activity	0.0044	*SERPINB5*, *SOCS3*, *PPP1R1A*, *CST1*, *SFN*, *CAMK2N1*
GO:0008201	Heparin binding	0.0081	*CCL2*, *CCL8*, *LAMC2*, *THBS1*
GO:0004222	Metalloendopeptidase activity	0.0083	*MMP19*, *MMP28*, *ADAMTS12*, *MMP11*
GO:0004860	Protein kinase inhibitor activity	0.0091	*SOCS3*, *SFN*, *CAMK2N1*

GO, Gene Ontology; DEGs, differentially expressed genes; BP, biological process; CC, cellular component; MF, molecular function.

Table IV. Virtual validation results of the common DEGs using 4 PDAC datasets.

Dataset	Samples	Genes found	Risk groups p-value	CI	DEGs between risk groups	
					No.	DEGs
TCGA	176	75	9.90E-15	79.6	49	*TMC5*, *GJB2*, *DPCR1*, *MMP11*, *ZWINT*…
ICGC	189	75	0.00E+00	82.1	17	*CCL8*, *ZWINT*, *NQO1*, *OAS1*, *ECT2*…
GSE21501	132	75	6.00E-13	95.4	11	*SLC6A14*, *CEACAM5*, *FOSB*, *LAMA3*, *ANO1*…
GSE28735	90	73	1.10E-06	99.9	4	*RRM2*, *PTGDS*, *ITGA2*, *C15orf48*

DEGs, differentially expressed genes; PDAC, pancreatic ductal adenocarcinoma; CI, concordance index.

cant molecular function were enriched for the common DEGs including structural molecule (p=0.0035), enzyme inhibitor (p=0.0044), heparin binding (p=0.0081), metalloendopeptidase (p=0.0083) and protein kinase inhibitor activities (p=0.0091) (Table III).

mRNA-miRNA network construction. To explore mRNA and miRNA regulation mechanism, the mRNA-miRNA pairings were constructed. The results indicated that the 5 miRNAs were able to target 3,760, 827 and 3,671 genes in the MicroCosm, mirTarbase and TargetScan database,

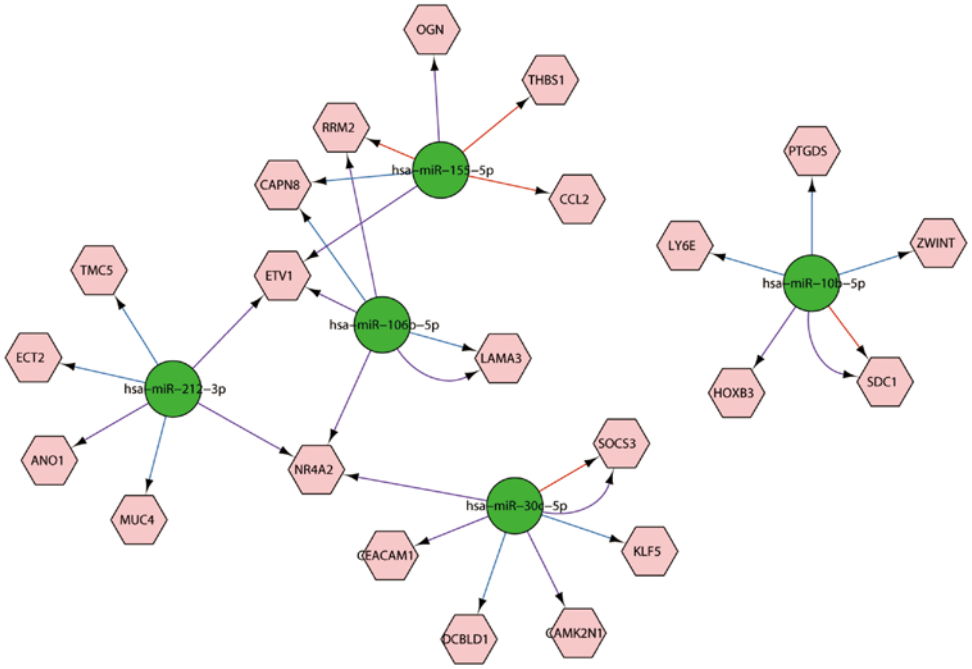

Figure 4. Interaction network between the common differentially expressed genes (DEGs) and 5 reported miRNAs. Pink hexagon represent DEGs and green circle represent miRNAs.

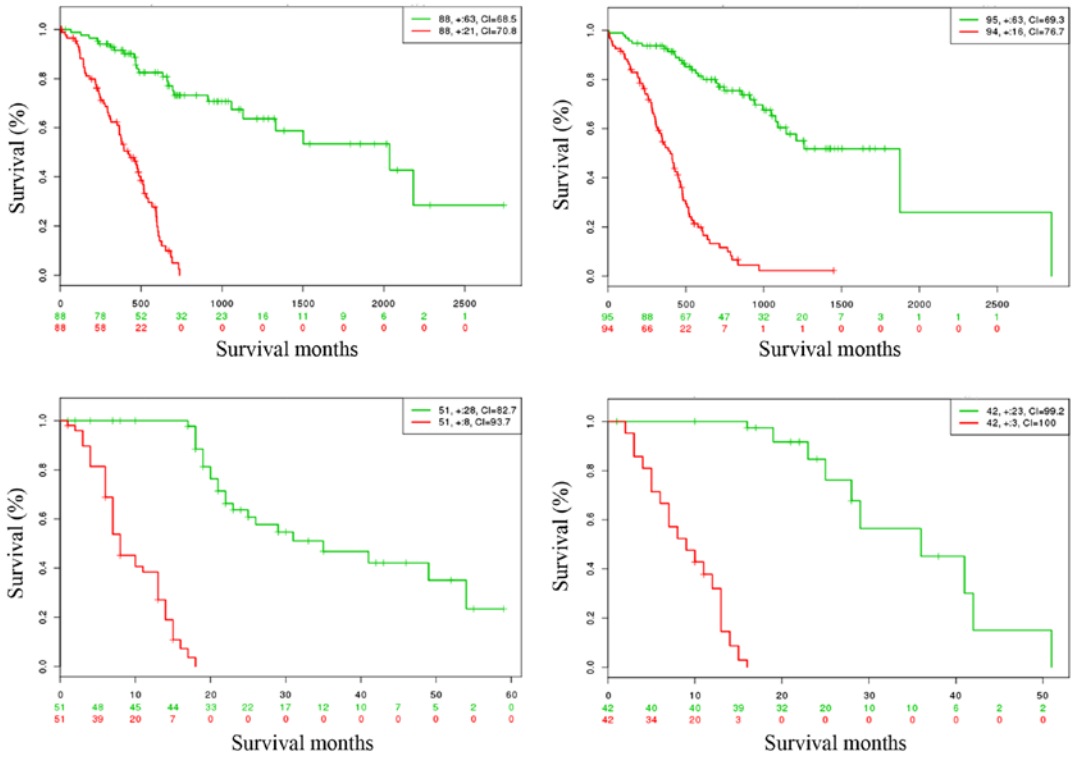

Figure 5. Kaplan-Meier curves of 4 bladder cancer datasets in SurvExpress database. Censored samples are marked with '+'. The x-axis represents time to event and y-axis represents percentage. The number of samples, censored number and concordance index (CI) are shown in the top-right insets. High- and low-risk groups are labeled with red and green curves, respectively.

respectively. Among those, 22 genes were also identified in the common DEGs. Then, the interaction network between the 22 genes and 5 miRNAs were constructed (Fig. 4). Based on the figure, we found that hsa-miR-212-3p can regulate 6 genes, hsa-miR-106p-5p can regulate 5 genes, hsa-miR-155-5p can regulate 5 genes, hsa-miR-10b-5p can regulate 5 genes and hsa-miR-30c-5p can regulate 6 genes.

Virtual validation. Biomarker validation is critical in the study of cancer molecular mechanism research. The prognostic

performance of the common DEGs were validated using the SurvExpress online tool which provides survival analysis and risk assessment. Results are shown in Fig. 5 and are summarized in Table IV. The figure indicated that low- and high-risk PDAC groups can be significantly differentiated in the 4 datasets, and the p-values were 9.9e-15, 0.0, 6.0e-13 and 1.1e-06, respectively. In addition, the higher concordance index (CI) demonstrated that better prediction results can be achieved based on those DEGs (Table IV).

Discussion

With the development of microarray and next generation sequencing technologies, understanding of the molecular mechanism of pancreatic ductal adenocarcinoma (PDAC) has been significantly advanced. In the present study, we aimed to unveil the complex mechanism of PDAC by integrating different types of omics data. A total of 76 genes were simultaneously differentially expressed in 39 tumor tissues. Notably, a heat map showed that tumor and normal samples were able to be clearly distinguished based on the DEGs. The erroneous assignment of 2 samples was probably due to tumor heterogeneity.

KEGG pathway enrichment revealed that the DEGs were involved in the ECM-receptor interaction pathway. Studies have shown that PDAC is characterized by prominent desmoplasia (24). Extracellular matrix (ECM) proteins, ECM metabolizing enzymes and growth factors are main components of desmoplasia, and the components can promote the growth of cancer cells (25). In addition, ECM proteins and desmoplastic secreted growth factors can activate intracellular signals including reactive oxygen species that prevent the death of PDAC cancer cells (25). In addition, several ECM components such as collagens I/III/IV, decorin and versican may be of clinical prognostic significance in PDAC (26). Another significantly enriched pathway is focal adhesion. Researches have demonstrated that interaction between integrin and focal adhesion kinase can regulate cancer cell adhesion and invasion (27,28). Sawai *et al* showed that phosphorylation of focal adhesion kinase is involved in the aggressive capability of PDAC via the Ras/ERK signaling pathway (29). In addition, MUC16, a heavily glycosylated type-I transmembrane mucin, can facilitate PDAC growth and metastasis via focal adhesion signaling (30).

The mRNA and miRNA interaction network analysis further unveiled the complex mechanism of PDAC. The interaction network showed that hsa-miR-212-3p can regulate the DEG *MUC4*. This gene encodes highly glycosylated integral membrane glycoprotein in the cell surface (31). In addition, immunohistochemical analyses based on 135 PDAC tissues demonstrated that *MUC4* was significantly highly expressed in patients with poor prognosis (p=0.0043) (32). In addition, hypomethylation of the *MUC4* promoter probably participated in the carcinogenesis and malignant development of PDAC based on DNA methylation-specific PCR analysis of 116 microdissected foci (33). Jonckheere *et al* showed that *MUC4* can interact with the ErbB2 oncogenic receptor via EGF domains, and inhibition of *MUC4* expression can affect the downstream JNK pathway (34).

Another important gene is *RRM2* which encodes one of 2 non-identical subunits for ribonucleotide reductase. Ribonucleotide reductase has been demonstrated to be a determinant of gemcitabine chemoresistance in human cancers (35), and the level of *RRM2* can regulate enzyme activity (36). Duxbury *et al* showed that high expression of *RRM2* is related to gemcitabine chemoresistance in PDAC (37). The suppression of *RRM2* by siRNA significantly inhibited tumor growth, metastasis and increased tumor apoptosis (37). Research has also shown that *NF-κB* is the key mediator by which *RRM2* induces invasiveness in PDAC (38). *RRM2* and its downstream intermediaries can become potential drug targets (38). The mRNA-miRNA interaction network demonstrated that hsa-miR-106p-5p and hsa-miR-155-5p can regulate *RRM2*.

In addition, the *CCL2* gene, which is also refered to as monocyte chemoattractant protein 1 and is a small cytokine (39), has been widely reported to be related to PDAC progression. In PDAC, the expression of *CCL2* was found to be significantly elevated (40). In addition, Kalbasi *et al* demonstrated that highly expressed *CCL2* can recruit $Ly6C^{+}CCR2^{+}$ inflammatory monocytes or macrophages to the regions surrounding the tumor and promote tumor proliferation and vascularization (41). In addition, PDAC cancer cells can construct an immunosuppressive tumor microenvironment by recruiting regulatory T cells with the high-expression of CCL5 (42). The mRNA-miRNA interaction network showed that hsa-miR-155-5p can regulate *CCL2*.

Apart from the above-mentioned 3 critical genes in PDAC, some rarely reported or novel genes such as *ECT2*, *SDC1*, *SOCS3*, *TMC5* and *NR4A2*, also play a role in the development of PDAC. The *ECT2* gene can promote Rho activity during cytokinesis, and RT-PCR results showed that it was highly expressed in PDAC (43). Statistical analysis showed that epithelial expression of *SDC1* was positively correlated with survival time in PDAC patients (p=0.029) (44). Lesina *et al* showed that homozygous deletion of *Socs3* can aberrantly activate *Stat3* and promote PDAC development (45).

In summary, the development and progression of PDAC were probably induced via various processes. Firstly, activation of *MUC4* induces nuclear translocation of β-catenin and promotes the process of angiogenesis that can provide necessary nutrition or oxygen for cancer cells. Then, *RRM2* can induce the invasiveness of PDAC via *NF-κB*. Finally, the formation of an immunosuppressive tumor microenvironment by recruiting regulatory T cells with high expression of *CCL2* further promotes cancer cell proliferation and vascularization.

Acknowledgements

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