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Integrated microRNA-mRNA analysis of pancreatic ductal adenocarcinoma

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ABSTRACT. The main aim of this study was to explore the underlying molecular mechanisms and potential target molecules of pancreatic adenocarcinoma. The miRNA (GSE32678) and mRNA (GSE32676) expression profiles of patients with pancreatic ductal adenocarcinoma and healthy controls were downloaded from the Gene Expression Omnibus database. Differentially expressed miRNA and differentially expressed genes were identified by analyzing the microarray algorithm

after data preprocessing. Functional analysis was conducted by the Database for Annotation, Visualization and Integrated Analysis. miRNA-mRNA regulation pairs were obtained in TarMir database. The node degree of *hsa-miR-200c*, *hsa-miR-429*, and *hsa-miR-200b* (miRNA), and *EFNB2*, *MYRIP*, and *PHF17* (mRNA) were extremely high in the miRNA-mRNA network, indicating that these miRNA and mRNA may play a key role in the development of pancreatic cancer. Our study screened out some target miRNAs and mRNAs for pancreatic ductal adenocarcinoma, which may be helpful in its diagnosis and treatment.

Key words: Pancreatic adenocarcinoma; MicroRNA; Differentially expressed gene

INTRODUCTION

Pancreatic adenocarcinoma is a highly lethal disease that is usually diagnosed at an advanced stage of disease progression. This type of cancer has very few or no effective therapies (Long et al., 2012; Siegel et al., 2014). This disease is characterized by an early local regional spread and distant metastasis. The mortality rate of pancreatic adenocarcinoma is almost 100%, because of its propensity for early metastatic spreading, and its resistance to radiation and chemotherapy. Despite the vast amount of accumulated knowledge regarding tumor biology, the efficacy of the available treatment strategies for pancreatic cancer has remained largely unchanged over the past decade. The first-line agent gemcitabine has been observed to show favorable clinical responses, including reduced pain and weight gain (Matano et al., 2000); despite this, the prognosis remains dismal, with a 5-year survival rate of 1-4%, and a median survival period of 4-6 months. In addition, the molecular basis for pancreatic cancer remains to be elucidated. Therefore, there is a need for further research at a molecular level to identify new molecular mechanisms or biomarkers, in an effort to improve the prognosis, diagnosis, and treatment of pancreatic cancer.

MicroRNAs (miRNA) are 18-24-nucleotide long non-coding RNA that bind to the 3'-untranslated region of target transcripts and regulate gene expression by degrading the target mRNA or inhibiting translation (Lee et al., 1993). Previous studies have indicated that miRNA serve as guidance molecules, base pairing with partial or full complementary sequences of target mRNA, leading to translational repression and/or mRNA cleavage. Research conducted to elucidate the mechanism of action of miRNA has revealed that miRNA affect stem cell differentiation, organ development, cell death, phase change in the cell cycle, signal transduction, and several diseases, including cancer (Lionetti et al., 2009; Antonini et al., 2010). Recent studies have focused on miRNA expression profiling of pancreatic adenocarcinoma. Aberrant expression of miRNA, such as miR-21 and miR-155, in the pancreas, often contributes to cancer development and invasion (Giovannetti et al., 2010; Ryu et al., 2010). On the other hand, miRNA such as miR-34 and miR-150 may also suppress the growth and malignant behavior of pancreatic cancer cells (Liu et al., 2011; Srivastava et al., 2011). Several research groups have performed bioinformatic analyses, including gene ontology annotation of molecular function, biological processes and cellular components, and Kyoto Encyclopedia

of Genes and Genomes (KEGG) pathways and predicted target genes, on mRNA or miRNA displaying altered expression. Despite these, research on the relationship between miRNA and the development of pancreatic adenocarcinoma using high throughput methodologies is extremely rare. This study provides important information to facilitate the elucidation of the physiological and pathological processes of pancreatic adenocarcinoma.

MATERIAL AND METHODS

mRNA and miRNA microarray data

GSE32676 and GSE32678, each containing 32 mRNA or miRNA expression chips from 25 early-stage pancreatic ductal adenocarcinoma (PDAC) samples and 7 non-malignant pancreatic samples, and uploaded by the same contributors, were downloaded from the gene expression omnibus (GEO) database.

Preprocessing of microarray data

mRNA and miRNA microarray data must be preprocessed prior to the identification of differentially expressed genes. Background correction and normalization was performed using the Affy package in R (R Core Team, 2013). Upon the detection of a gene by multiple probes, the mean expression value of those probes was utilized.

Identification of differentially expressed genes and miRNA

The Limma package in the R programming platform was used to identify the differentially expressed genes (DEGs) and miRNA. The Student *t*-test and the Bonferroni's correction method was used with an adjusted P value <0.05 and a $|\log FC|$ value >1.

Functional annotation of DEGs

Functional annotation of the DEGs was performed using the Database for Annotation, Visualization and Integrated Discovery (http://david.abcc.ncifcrf.gov/, DAVID). Gene ontology (GO) terms and KEGG pathways with P values <0.05 and at least 5 genes were selected.

Screening of miRNA-mRNA relationships

The miRNA-mRNA relationships were screened using the TarMir (http://www.tarmir.rgcb.res.in/) database. TarMir integrates a majority of the common miRNA databases, such as TargetScan, miRanda, and Point-in-Time Architecture (PITA), to identify the miRNA-mRNA relationships in a customizable and comprehensive manner. In this method, the miRNA-mRNA relationships identified in DIANA, miRanda, PITA, and TargetScan were simultaneously retrieved by TarMir, the overlapped genes between DEGs and the target genes of differentially expressed miRNA were filtered, and the miRNA-mRNA regulatory network was constructed using those miRNA-mRNA relationships. The network was visualized using the Cytoscape platform software (Shannon et al., 2003).

RESULTS

Preprocessing of microarray data and identification of DEGs

Normalized gene and miRNA expression profiles were obtained after background correction and the normalization of microarray data. The box plots of gene expression data before and after preprocessing are shown in Figure 1. Six hundred and twenty DEGs, including 441 up-regulated and 179 down-regulated genes, were identified by the Student *t*-test and Bonferroni's correction. Forty-eight differentially expressed miRNA (27 up-regulated and 21 down-regulated miRNA) were also selected.

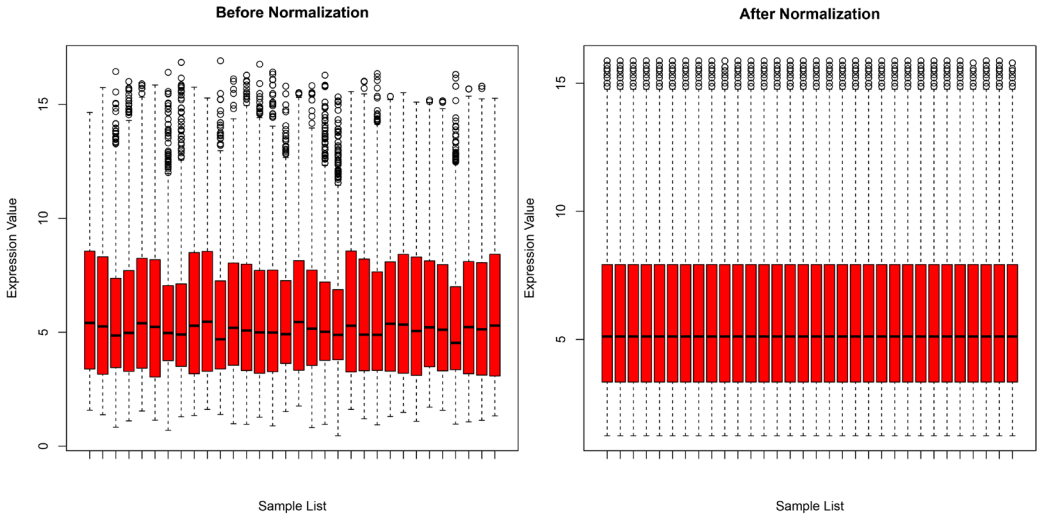

Figure 1. Expression value of all genes in every sample before and after normalization.

Functional annotation of DEGs

A total of 111 GO terms, such as wound healing, cell adhesion, and biological adhesion, were enriched in the DEGs. In addition, 11 KEGG pathways, containing cell adhesion molecules, small cell lung cancer, and the p53 signaling pathway, which are well-researched aspects of cancers, were found to be significant (Table 1).

miRNA-mRNA relationship

Six thousand and nine hundred and thirty-one miRNA-mRNA regulatory relationships were retrieved from the TarMir database. Among these, 220 relationships were defined by DEGs and differentially expressed miRNA (Table 2). These were used to construct an miRNA-mRNA regulatory network (Figure 2) in PDAC. Corresponding degrees of genes/miRNAs in the miRNA-mRNA regulatory network are shown in Table 3.

Table 1. KEGG pathways of differentially expressed genes (DEGs).

Category	Pathway name	No. of genes	P value	Genes
KEGG_PATHWAY	O-glycan biosynthesis	7	4.02E-04	*GALNT3*, *GCNT3*, *GALNT7*, *GALNT5*, *GALNT12*, *C1GALT1*, *ST6GALNAC1*
KEGG_PATHWAY	Cell adhesion molecules	11	0.012884	*F11R*, *CLDN7*, *SDC1*, *PTPRF*, *ITGA6*, *CLDN1*, *CDH1*, *CDH3*, *SDC4*, *SELE*, *CLDN23*
KEGG_PATHWAY	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	8	0.013361	*JUP*, *DSG2*, *ITGA6*, *ITGB6*, *ITGA2*, *CACNB3*, *ITGA3*, *TCF7L2*
KEGG_PATHWAY	Tight junction	11	0.014215	*F11R*, *EPB41L3*, *CLDN7*, *CTTN*, *CGN*, *CLDN1*, *PRKCI*, *MYH14*, *TJP3*, *CLDN23*, *LLGL2*
KEGG_PATHWAY	Small cell lung cancer	8	0.022157	*LAMB3*, *CCND1*, *ITGA6*, *PIAS3*, *ITGA2*, *LAMC2*, *ITGA3*, *MYC*
KEGG_PATHWAY	ECM-receptor interaction	8	0.022157	*LAMB3*, *SDC1*, *ITGA6, ITGB6*, *ITGA2*, *LAMC2*, *ITGA3*, *SDC4*
KEGG_PATHWAY	p53 signaling pathway	7	0.025923	*TP53I3*, *CCND1*, *SERPINB5*, *RRM2*, *SFN*, *PERP*, *GADD45B*
KEGG_PATHWAY	Pathways in cancer	21	0.026051	*WNT5A*, *BMP4*, *IL6*, *EGLN3*, *ITGA2*, *EGLN3*, *ITGA2*, *CDH1*, *ITGA3*, *ZBTB16*, *MECOM*, *TCF7L2*, *JUP, CBLC*, *LAMB3*, *CCND1*, *ITGA6*, *PIAS3*, *LAMC2*, *MYC*, *GSTP1*
KEGG_PATHWAY	Jak-STAT signaling pathway	11	0.03508	*CSF3*, *CBLC*, *CCND1*, *IL6*, *SOCS2*, *PIAS3*, *SOCS3*, *PIM1*, *LIFR*, *MYC*, *GHR*
KEGG_PATHWAY	Acute myeloid leukemia	6	0.044307	*JUP*, *CCND1*, *PIM1*, *ZBTB16*, *MYC*, *TCF7L2*
KEGG_PATHWAY	Aldosterone-regulated sodium reabsorption	5	0.047589	*ATP1B1*, *SGK1*, *SFN*, *SCNN1A*, *IRS1*

DISCUSSION

The pathogenesis of pancreatic adenocarcinoma remains poorly understood, which allows for the development of novel preventive and therapeutic interventions. In this study, the miRNA and mRNA expression profiles of patients with early-stage PDAC, as well as those of non-malignant pancreatic samples, were downloaded from gene databases. The miRNA (GSE32678) and mRNA (GSE32676) array deposited in the GEO database was analyzed, resulting in the identification of 620 DEGs from the original dataset, including 441 up-regulated and 179 down-regulated genes.

Table 2. miRNA-mRNA regulatory relationship comprised of differentially expressed genes and differentially expressed microRNA (miRNA).

miRNA	mRNA	miRNA	mRNA	miRNA	mRNA	miRNA	mRNA
hsa-miR-135b	*ADAMTS9*	*hsa-miR-429*	*TFAP2A*	*hsa-miR-182*	*CTTN*	*hsa-miR-34a*	*JAKMIP1*
hsa-miR-135b	*CALML4*	*hsa-miR-429*	*TNFSF8*	*hsa-miR-182*	*EFNB2*	*hsa-miR-34a*	*MARCKSL1*
hsa-miR-135b	*CDR2L*	*hsa-miR-425*	*CREM*	*hsa-miR-182*	*ELMO1*	*hsa-miR-34a*	*MYRIP*
hsa-miR-135b	*EFNB2*	*hsa-miR-425*	*EFNB2*	*hsa-miR-182*	*FGR*	*hsa-miR-34a*	*NHS*
hsa-miR-135b	*EMP1*	*hsa-miR-425*	*MYRIP*	*hsa-miR-182*	*FNBP1L*	*hsa-miR-34a*	*NR4A2*
hsa-miR-135b	*GHR*	*hsa-miR-425*	*SH3RF1*	*hsa-miR-182*	*KIAA1217*	*hsa-miR-34a*	*NRN1*
hsa-miR-135b	*PHF17*	*hsa-miR-222*	*CEP55*	*hsa-miR-182*	*MYRIP*	*hsa-miR-34a*	*SOX4*
hsa-miR-135b	*RAB39B*	*hsa-miR-222*	*EFNB2*	*hsa-miR-182*	*NHS*	*hsa-miR-28-5p*	*ANK2*
hsa-miR-135b	*TRPM4*	*hsa-miR-222*	*NAP1L2*	*hsa-miR-182*	*NRN1*	*hsa-miR-28-5p*	*BZRAP1*
hsa-miR-200a	*DCUN1D3*	*hsa-miR-222*	*NAP1L5*	*hsa-miR-182*	*TOX3*	*hsa-miR-28-5p*	*DCLK1*
hsa-miR-200a	*FAM46C*	*hsa-miR-222*	*PAK1*	*hsa-miR-874*	*ARHGAP12*	*hsa-miR-28-5p*	*EFNA5*
hsa-miR-200a	*FNBP1L*	*hsa-miR-489*	*EFNA5*	*hsa-miR-874*	*DCUN1D3*	*hsa-miR-28-5p*	*HOXB3*
hsa-miR-200a	*HOXB5*	*hsa-miR-489*	*NFIL3*	*hsa-miR-874*	*NHS*	*hsa-miR-28-5p*	*NR4A3*
hsa-miR-200a	*ITGA6*	*hsa-miR-489*	*NR4A3*	*hsa-miR-96*	*ANLN*	*hsa-miR-28-5p*	*PLEKHA7*
hsa-miR-200a	*MYRIP*	*hsa-miR-203*	*ACSL1*	*hsa-miR-96*	*CAMK2N1*	*hsa-miR-28-5p*	*S1PR1*
hsa-miR-200a	*RAB27B*	*hsa-miR-203*	*DCUN1D3*	*hsa-miR-96*	*FOXQ1*	*hsa-miR-28-5p*	*SLC4A11*
hsa-miR-200a	*RHPN2*	*hsa-miR-203*	*DPY19L2*	*hsa-miR-96*	*GRHL2*	*hsa-miR-488*	*ADAM9*
hsa-miR-200a	*TFAP2A*	*hsa-miR-203*	*FKBP1B*	*hsa-miR-96*	*IRS1*	*hsa-miR-488*	*ADRB2*
hsa-miR-200a	*WNT5A*	*hsa-miR-203*	*IRS1*	*hsa-miR-96*	*MYRIP*	*hsa-miR-488*	*KCNS3*
hsa-miR-200b	*ADAMTS3*	*hsa-miR-203*	*ITGA2*	*hsa-miR-96*	*NR4A3*	*hsa-miR-488*	*KIAA1217*
hsa-miR-200b	*ANLN*	*hsa-miR-203*	*NFIL3*	*hsa-miR-96*	*PAK1*	*hsa-miR-488*	*MXRA5*
hsa-miR-200b	*CDR2L*	*hsa-miR-203*	*NR4A3*	*hsa-miR-96*	*PROK2*	*hsa-miR-488*	*PPAP2B*
hsa-miR-200b	*EFNA1*	*hsa-miR-203*	*PPAP2B*	*hsa-miR-96*	*RHPN2*	*hsa-miR-488*	*SH2B3*
hsa-miR-200b	*EFNB2*	*hsa-miR-203*	*PREX1*	*hsa-miR-135a*	*ADAMTS9*	*hsa-miR-488*	*STK39*
hsa-miR-200b	*LMO7*	*hsa-miR-203*	*SOCS3*	*hsa-miR-135a*	*CALML4*	*hsa-miR-526a*	*ANK2*
hsa-miR-200b	*LRRC8A*	*hsa-miR-203*	*TOX3*	*hsa-miR-135a*	*CDR2L*	*hsa-miR-526a*	*EFNB2*
hsa-miR-200b	*MBOAT2*	*hsa-miR-608*	*EBF1*	*hsa-miR-135a*	*EFNB2*	*hsa-miR-526a*	*EGLN3*
hsa-miR-200b	*NAP1L2*	*hsa-miR-608*	*NRN1*	*hsa-miR-135a*	*EMP1*	*hsa-miR-526a*	*HOXB3*
hsa-miR-200b	*NAP1L5*	*hsa-miR-608*	*PIP4K2C*	*hsa-miR-135a*	*GHR*	*hsa-miR-141*	*DCUN1D3*
hsa-miR-200b	*PHF17*	*hsa-miR-608*	*RAB39B*	*hsa-miR-135a*	*PHF17*	*hsa-miR-141*	*FAM46C*
hsa-miR-200b	*PPAP2B*	*hsa-miR-183*	*ENC1*	*hsa-miR-135a*	*RAB39B*	*hsa-miR-141*	*FNBP1L*
hsa-miR-200b	*PROK2*	*hsa-miR-183*	*IRS1*	*hsa-miR-135a*	*TRPM4*	*hsa-miR-141*	*HOXB5*
hsa-miR-200b	*PVRL4*	*hsa-miR-183*	*KIAA0101*	*hsa-miR-34c-5p*	*ACSL1*	*hsa-miR-141*	*ITGA6*
hsa-miR-200b	*TFAP2A*	*hsa-miR-183*	*PIM1*	*hsa-miR-34c-5p*	*ACSL4*	*hsa-miR-141*	*MYRIP*
hsa-miR-200b	*TNFSF8*	*hsa-miR-183*	*TCF7L2*	*hsa-miR-34c-5p*	*ANK2*	*hsa-miR-141*	*RAB27B*
hsa-miR-21	*GRAMD3*	*hsa-miR-200c*	*ADAMTS3*	*hsa-miR-34c-5p*	*CBFA2T3*	*hsa-miR-141*	*RHPN2*
hsa-miR-21	*LIFR*	*hsa-miR-200c*	*ANLN*	*hsa-miR-34c-5p*	*DKK1*	*hsa-miR-141*	*SOX9*
hsa-miR-21	*MYO6*	*hsa-miR-200c*	*CDR2L*	*hsa-miR-34c-5p*	*FKBP1B*	*hsa-miR-141*	*TFAP2A*
hsa-miR-21	*PHF17*	*hsa-miR-200c*	*EFNA1*	*hsa-miR-34c-5p*	*FOXQ1*	*hsa-miR-141*	*WNT5A*
hsa-miR-21	*PLEKHA1*	*hsa-miR-200c*	*EFNB2*	*hsa-miR-34c-5p*	*GALNT7*	*hsa-miR-92b*	*ADAM10*
hsa-miR-21	*SASH1*	*hsa-miR-200c*	*LMO7*	*hsa-miR-34c-5p*	*JAKMIP1*	*hsa-miR-92b*	*CNNM4*
hsa-miR-429	*ADAMTS3*	*hsa-miR-200c*	*LRRC8A*	*hsa-miR-34c-5p*	*MARCKSL1*	*hsa-miR-92b*	*DNAJB9*
hsa-miR-429	*ANLN*	*hsa-miR-200c*	*MBOAT2*	*hsa-miR-34c-5p*	*MYRIP*	*hsa-miR-92b*	*EDNRB*
hsa-miR-429	*CDR2L*	*hsa-miR-200c*	*NAP1L2*	*hsa-miR-34c-5p*	*NHS*	*hsa-miR-92b*	*GALNT7*
hsa-miR-429	*EFNA1*	*hsa-miR-200c*	*NAP1L5*	*hsa-miR-34c-5p*	*NR4A2*	*hsa-miR-92b*	*GHR*
hsa-miR-429	*EFNB2*	*hsa-miR-200c*	*PHF17*	*hsa-miR-34c-5p*	*NRN1*	*hsa-miR-92b*	*GRAMD3*
hsa-miR-429	*LMO7*	*hsa-miR-200c*	*PPAP2B*	*hsa-miR-34c-5p*	*SOX4*	*hsa-miR-92b*	*NR4A3*
hsa-miR-429	*LRRC8A*	*hsa-miR-200c*	*PROK2*	*hsa-miR-34a*	*ACSL1*	*hsa-miR-92b*	*PLEKHA1*
hsa-miR-429	*MBOAT2*	*hsa-miR-200c*	*PVRL4*	*hsa-miR-34a*	*ACSL4*	*hsa-miR-92b*	*PPAP2B*
hsa-miR-429	*NAP1L2*	*hsa-miR-200c*	*TFAP2A*	*hsa-miR-34a*	*ANK2*	*hsa-miR-92b*	*S1PR1*
hsa-miR-429	*NAP1L5*	*hsa-miR-200c*	*TNFSF8*	*hsa-miR-34a*	*CBFA2T3*	*hsa-miR-92b*	*SOX4*
hsa-miR-429	*PHF17*	*hsa-miR-671-5p*	*PHF17*	*hsa-miR-34a*	*DKK1*	*hsa-miR-92b*	*STK39*
hsa-miR-429	*PPAP2B*	*hsa-miR-182*	*ADAM10*	*hsa-miR-34a*	*FKBP1B*	*hsa-miR-552*	*DCUN1D3*
hsa-miR-429	*PROK2*	*hsa-miR-182*	*ANK2*	*hsa-miR-34a*	*FOXQ1*	*hsa-miR-552*	*ENC1*
hsa-miR-429	*PVRL4*	*hsa-miR-182*	*CBFA2T3*	*hsa-miR-34a*	*GALNT7*	*hsa-miR-552*	*SGK1*

GO analysis revealed many significantly enriched biological processes, including wound response and healing, cell adhesion, biological adhesion, inflammatory response, and response to corticosteroid stimulus. The KEGG pathway-enrichment analysis revealed a sig-

nificant overexpression of the o-glycan biosynthesis pathway, cell adhesion molecules, arrhythmogenic right ventricular cardiomyopathy, and tight junction, in the early-stage PDAC samples. Pancreatic ductal adenocarcinoma is characterized by early loco-regional spread and distant metastasis. The development of metastasis is determined by the gradual increase in essential changes in cancerous cells, as well as their communications with different stromal elements in the tumor microenvironment. Cell adhesion and biological adhesion play significant roles in cancer metastasis. Moreover, the inflammatory response and the migration of the myeloid (macrophages, dendritic cells, neutrophils, myeloid-derived suppressor cells) and lymphoid (regulatory T, B and NK cells) immune regulatory cells to the tumor site have been reported to support tumor growth, spread of tumor, and tumor metastasis (Keskinov and Shurin, 2014).

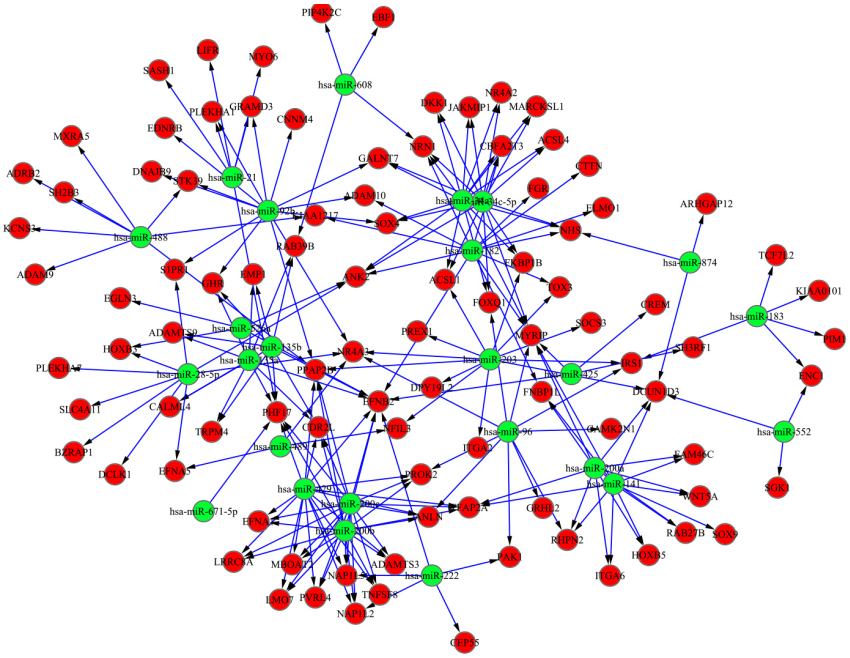

Figure 2. miRNA-mRNA regulation network. Red nodes represent genes and bright green nodes represent miRNAs.

The target genes regulated by the differentially expressed miRNA and mRNA were then identified and further analyzed, in order to define their relationship with miRNA and mRNA; subsequently, an miRNA-mRNA regulatory network was constructed.

A thorough analysis of the regulatory network revealed an extremely high node degree of *hsa-miR-200c*, *hsa-miR-429*, *and hsa-miR-200b* (miRNA), and *EFNB2*, *MYRIP*, and *PHF17* (mRNA), which indicated that these miRNA and mRNA may play a key role in the development of pancreatic cancer. The miR-200 family (*miR-200a*, *miR-200b*, *miR-200c*, *miR-141*, and *miR-429*) is a cluster of miRNA that are highly correlated with epithelial-mesenchymal transition, with *miR-200b* being identified as a critical regulator of tumor invasion,

Table 3. Genes/microRNA (miRNA) in the miRNA-mRNA regulatory network and their corresponding degree.

Node	Degree	Node	Degree
hsa-miR-671-5p	1	*HOXB3*	2
LIFR	1	*S1PR1*	2
MYO6	1	*STK39*	2
SASH1	1	*hsa-miR-489*	3
CREM	1	*hsa-miR-874*	3
SH3RF1	1	*hsa-miR-552*	3
CEP55	1	*GHR*	3
DPY19L2	1	*RAB39B*	3
ITGA2	1	*FNBP1L*	3
PREX1	1	*RHPN2*	3
SOCS3	1	*ADAMTS3*	3
EBF1	1	*EFNA1*	3
PIP4K2C	1	*LMO7*	3
KIAA0101	1	*LRRC8A*	3
PIM1	1	*MBOAT2*	3
TCF7L2	1	*PVRL4*	3
CTTN	1	*TNFSF8*	3
ELMO1	1	*ACSL1*	3
FGR	1	*FKBP1B*	3
ARHGAP12	1	*IRS1*	3
CAMK2N1	1	*CBFA2T3*	3
GRHL2	1	*FOXQ1*	3
BZRAP1	1	*GALNT7*	3
DCLK1	1	*SOX4*	3
PLEKHA7	1	*hsa-miR-425*	4
SLC4A11	1	*hsa-miR-608*	4
ADAM9	1	*hsa-miR-526a*	4
ADRB2	1	*ANLN*	4
KCNS3	1	*NAP1L2*	4
MXRA5	1	*NAP1L5*	4
SH2B3	1	*PROK2*	4
EGLN3	1	*NRN1*	4
SOX9	1	*NHS*	4
CNNM4	1	*hsa-miR-222*	5
DNAJB9	1	*hsa-miR-183*	5
EDNRB	1	*CDR2L*	5
SGK1	1	*DCUN1D3*	5
ADAMTS9	2	*TFAP2A*	5
CALML4	2	*NR4A3*	5
EMP1	2	*ANK2*	5
TRPM4	2	*hsa-miR-21*	6
FAM46C	2	*PPAP2B*	6
HOXB5	2	*PHF17*	7
ITGA6	2	*MYRIP*	7
RAB27B	2	*hsa-miR-488*	8
WNT5A	2	*hsa-miR-135b*	9
GRAMD3	2	*hsa-miR-135a*	9
PLEKHA1	2	*hsa-miR-28-5p*	9
PAK1	2	*EFNB2*	9
EFNA5	2	*hsa-miR-200a*	10
NFIL3	2	*hsa-miR-96*	10
TOX3	2	*hsa-miR-141*	11
ENC1	2	*hsa-miR-203*	12
ADAM10	2	*hsa-miR-182*	13
KIAA1217	2	*hsa-miR-92b*	13
ACSL4	2	*hsa-miR-34c-5p*	15
DKK1	2	*hsa-miR-34a*	15
JAKMIP1	2	*hsa-miR-200b*	16
MARCKSL1	2	*hsa-miR-429*	16
NR4A2	2	*hsa-miR-200c*	16

The degree of genes is the number of miRNA that directly interact with it, while the degree of miRNA is denoted by the number of genes that directly interact with it.

metastasis, and chemosensitivity (Feng et al., 2012). The miR-200 family is associated with the acquisition of epithelial-to-mesenchymal transition and gemcitabine sensitivity in pancreatic adenocarcinoma (Li et al., 2009; Ali et al., 2010; Bao et al., 2011). Previous studies have reported the close association of *EFNB2* with the development of gastric cancer, neuroblastomas, esophageal squamous cell carcinoma, and other tumors (Tang et al., 1999, 2000; Kataoka et al., 2002; Tachibana et al., 2007). However, the relationship between specific genes and pancreatic adenocarcinoma has been rarely reported. We believe that these genes could be potential targets for the diagnosis and treatment of pancreatic adenocarcinoma. In summary, this study provides important information to facilitate the elucidation of the physiological and pathological processes governing pancreatic adenocarcinoma. However, the roles played by these genes in pancreatic adenocarcinoma must be further validated *in vitro* and *in vivo*, using the latest molecular biology techniques.

Conflicts of interest

The authors declare no conflict of interest.

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