

Original Article

Potential gene and microRNA biomarkers for pancreatic cancer with gene expression analysis

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Abstract: Objective: This study aimed to reveal the underlying molecular mechanisms in the development of pancreatic cancer. Materials and methods: The gene expression profile of GSE32676 was downloaded from Gene Expression Omnibus, including 25 human pancreatic cancer samples and 7 non-malignant pancreas samples. The differentially expressed genes (DEGs) were firstly selected with two sample *t*-test. Then, pathway and functional enrichment analysis was performed for the identified up- and down-regulated DEGs. What's more, the miRNA-target regulatory network was constructed based on the experimentally validated regulatory relationships between DEGs and miRNAs from miRecords database. Results: Total 1113 DEGs were obtained, including 697 up-regulated and 416 down-regulated genes. The up- and down-regulated DEGs were significantly associated with the adhesion and immune response, respectively. In addition, 4 miRNAs including hsa-miR-145, hsa-miR-191, hsa-miR-125b and hsa-miR-124 were selected for the up-regulated DEGs, while 1 miRNA hsa-miR-185 was enriched for down-regulated DEGs (NTRK3 and CORO2B). Conclusions: The identified DEGs and enriched miRNAs regulating up- and down-regulated DEGs might play important roles in the development of pancreatic cancer, and the enriched miRNAs might participate in the development of pancreatic cancer by regulating the identified target DEGs.

Keywords: Pancreatic cancer, enrichment analysis, miRNA, regulatory network

Introduction

Pancreatic cancer is the fourth most common cause of cancer-related deaths worldwide and most patients are diagnosed at an advanced stage usually associated with poor prognosis as pancreatic cancer is asymptomatic in early stages [1-3]. Though surgical resection and adjuvant therapy improve the long-term survival, only about 10% of patients are eligible for the operation and treatment failures may occur due to local recurrence and hepatic metastases after surgery [4, 5]. Furthermore, there are no effective treatments for advanced pancreatic cancer, including local and metastatic diseases [6]. Infiltrating tumor cells are important elements in the pancreatic tumor microenvironment among which macrophages participate tumor progression and metastases [7]. It is urgently needed to discover the underlying molecular mechanisms of pancreatic cancer

which might contribute developing novel and effective strategies for pancreatic cancer.

In recent years, many genes and signaling pathways related to the development of pancreatic cancer have been investigated. The Notch pathway is involved in pancreatic tumorigenesis through a TGF (transforming growth factor)- α -mediated mechanism and γ -secretase inhibitor inducing Notch pathway inhibition might be used as a new therapeutic strategy for pancreatic cancer [8-10]. The Hedgehog signaling pathway also plays an important role pancreatic cancer invasion and metastasis [11]. Activation of signal transducer and activator of transcription 3 (STAT3) is usually occurred in several precancerous lesions and STAT3 inhibitors have the potential to be used for pancreatic cancer treatment since the expression of vascular endothelial growth factor (VEGF) and matrix metalloproteinase-2 (MMP-2) could be

down-regulated by silencing the STAT3 gene [12, 13]. The expression of NTRK3 (neurotrophin tyrosine kinase receptor 3) which is a neurotrophin receptor family member including NTRK1 (TRKA), NTRK2 (TRKB) and NTRK3 (TRKC) can activate the MAPK (mitogen activated protein kinase) and PI3K (phosphoinositide 3-kinase) pathways to promote cell differentiation and affect tumor progression [14, 15]. CORO2B (coronin, actin binding protein, 2B) is related to the neuronal actin cytoskeleton reorganization and cell migration [16, 17]. The specific roles of these two genes in the pathogenesis of pancreatic cancer are largely unclear. Meanwhile, the microRNAs (miRNAs) which could be used as candidate biomarkers for pancreatic cancer have been also studied extensively. Wan et al. have concluded that microRNA assay is very critical for the diagnosis of pancreatic cancer [18]. The miRNA-21 has been detected to be significantly overexpressed in pancreatic cancer [19], and miRNA-34 may be related to pancreatic cancer stem cell self-renewal by directly regulating downstream targets Notch and Bcl-2 [20]. The epigenetic silencing of miRNA-107 could inhibit pancreatic cancer growth accompanied by repression in cyclin-dependent kinase 6 (CDK6) level [21]. However, the molecular mechanisms of pancreatic cancer are not fully understood.

Therefore, the gene expression profiles of human pancreatic cancer and non-malignant pancreas samples were downloaded from Gene Expression Omnibus. The differentially expressed genes (DEGs) in pancreatic cancer were selected for pathway and function enrichment analysis. What's more, miRNA-target regulatory network was constructed based on the relationships of significantly enriched miRNAs and their corresponding up- and down-regulated target genes.

Materials and methods

Gene expression profile

The expression profile of GSE32676 [22] including 25 human pancreatic cancer samples with tumor cell content > 30% and 7 non-malignant pancreas (control) samples snap frozen at the time of surgery based on the platform of GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array, was downloaded from Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>). Meanwhile, the platform probe annotation information files were also downloaded.

Regulatory relation between miRNA and target genes was downloaded from miRTarBase [23] at April 5th, 2012. MiRTarBase database have integrated the regulatory relation verified by experiments, and there are total of 2862 regulatory relations, including 285 miRNAs and 1721 target genes.

Selection of differentially expressed genes

With Affy package of R language, data processing was performed. The probe corresponding to multiple gene symbols was deleted and average value of gene symbol with multiple probes was obtained for further analysis. Finally, the expression profile dataset including 19803 genes for the 32 samples were obtained. We used two sample *t*-test to analyze the differences in the gene expression between pancreatic cancer and control samples, and performed multiple testing corrections with Benjamin and Hochberg (BH) [24] method. The differentially expressed genes were selected with the cut-off criterion of false discovery rate (FDR) < 0.05.

Pathway enrichment analysis for DEGs

In order to recognize the biological functions involved with DEGs, we used hypergeometric distribution to perform Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis [25]. Assuming the KEGG pathways contain N genes and K DEGs, and the special pathway contains M genes. The probability of at least overlapping i genes can be calculated according to the following formula:

$$p = 1 - \sum_{i=0}^{x-1} \frac{\binom{M}{i} \binom{N-M}{K-i}}{\binom{N}{K}}$$

Moreover, Gene Ontology (GO) enrichment for interesting genes was made by DAVID (Database for Annotation, Visualization and Integrated Discovery) [26] which is a bioinformatics resource containing an integrated biological knowledgebase and analytic tools for extracting biological meaning from large lists of genes or proteins.

Construction of miRNA-target regulatory network

The experimentally validated regulatory relationships between DEGs and miRNAs were selected from miRecords database (<http://mirecords.org/>).

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Table 1. The significant KEGG pathways enriched for the identified DEGs

Path ID	Path Name	Total-gene	DEGs	P-value	FDR	Up-gene	Down-gene
Hsa04530	Tight junction	128	24	3.48E-06	0.00087	22	2
Hsa05144	Malaria	49	12	7.11E-05	0.00658	3	9
Hsa00512	Mucin type O-Glycan biosynthesis	29	9	7.93E-05	0.00658	8	1
Hsa04520	Adherens junction	72	14	0.00026	0.01631	13	1

Table 2. The pathway enrichment analysis for the identified up- and down-regulated DEGs

	Path ID	Path Name	Total-gene	DEGs	P-value	FDR
Up-regulated DEGs	Hsa04530	Tight junction	128	22	1.44e-08	3.58e-06
	Hsa04520	Adherens junction	72	13	8.27e-06	0.00103
	Hsa00512	Mucin type O-Glycan biosynthesis	29	9	1.90e-05	0.00158
	Hsa05200	Pathways in cancer	325	30	4.05e-05	0.00252
	Hsa04510	Focal adhesion	196	21	7.70e-05	0.00383
	Hsa05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	73	11	0.00023	0.00946
	Hsa04670	Leukocyte transendothelial migration	110	13	0.00073	0.02599
	Hsa05222	Small cell lung cancer	86	11	0.00096	0.02993
Down-regulated DEGs	Hsa05144	Malaria	49	9	2.55e-06	0.00064
	Hsa04630	Jak-STAT signaling pathway	149	13	7.62e-05	0.00948

cords.biolead.org/) [27]. The selected regulatory relations were used to construct the miRNA-target regulatory network. Firstly, the identified DEGs were divided into up-regulated genes and down-regulated genes. Then miRNAs were identified for the up-regulated and down-regulated DEGs with hypergeometric distribution, respectively. The miRNAs with P -value < 0.05 were considered to play roles in the pancreatic ductal adenocarcinoma by regulating corresponding DEGs. The p -value for having at least overlapping i differentially expressed target genes was calculated as the following formula:

$$p = 1 - \sum_{i=0}^{x-1} \frac{\binom{M}{i} \binom{N-M}{K-i}}{\binom{N}{K}}$$

Where the N represents the number of target genes in the regulatory relationships between DEGs and miRNAs; K represents the number of differentially expressed target genes; M represents the number of target genes of one miRNA.

Results

Extraction of differentially expressed genes

With the threshold of $FDR < 0.05$, 1113 differentially expressed genes were obtained, including 697 (62.62%) up-regulated genes and 416 (37.38%) down-regulated genes.

Pathway enrichment analysis for DEGs

The KEGG pathways enrichment analysis was performed for the identified DEGs with the threshold of $FDR < 0.5$, and total 4 significant pathways including Tight junction ($FDR = 0.00087$), Malaria ($FDR = 0.00658$), Mucin type O-Glycan biosynthesis ($FDR = 0.00658$) and Adherens junction ($FDR = 0.01631$) were selected (**Table 1**).

The up-regulated DEGs were significantly enriched in 8 pathways, such as Tight junction ($FDR = 3.58e-06$), Adherens junction ($FDR = 0.00103$) and Mucin type O-Glycan biosynthesis ($FDR = 0.00158$). Meanwhile, 2 significant pathways including Malaria ($FDR = 0.00064$) and Jak-STAT signaling pathway ($FDR = 0.00948$) were identified for down-regulated DEGs (**Table 2**).

Functional enrichment analysis for DEGs

The GO functional enrichment analysis for up- and down-regulated DEGs was conducted by DAVID with the cut-off criterion of $FDR < 0.05$. Total 3 and 5 significant functions were identified for the up- and down-regulated DEGs respectively (**Table 3**). The up-regulated DEGs were significantly related with the cell adhesion ($FDR = 6.03E-04$), biological adhesion ($FDR = 6.13E-04$) and ectoderm development ($FDR = 0.21858$). Meanwhile, 5 significant functions

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Table 3. The GO functional enrichment analysis for the identified up- and down-regulated DEGs

	Term	DEGs	P-value	FDR
Up-regulated DEGs	GO: 0007155~cell adhesion	54	3.47E-07	6.03E-04
	GO: 0022610~biological adhesion	54	3.53E-07	6.13E-04
	GO: 0007398~ectoderm development	22	1.26E-05	0.021858
Down-regulated DEGs	GO: 0006952~defense response	41	3.81E-10	6.58E-07
	GO: 0006954~inflammatory response	29	4.36E-10	7.54E-07
	GO: 0009611~response to wounding	36	3.55E-09	6.14E-06
	GO: 0055080~cation homeostasis	20	1.45E-05	0.02514
	GO: 0019221~cytokine-mediated signaling pathway	10	1.84E-05	0.031872

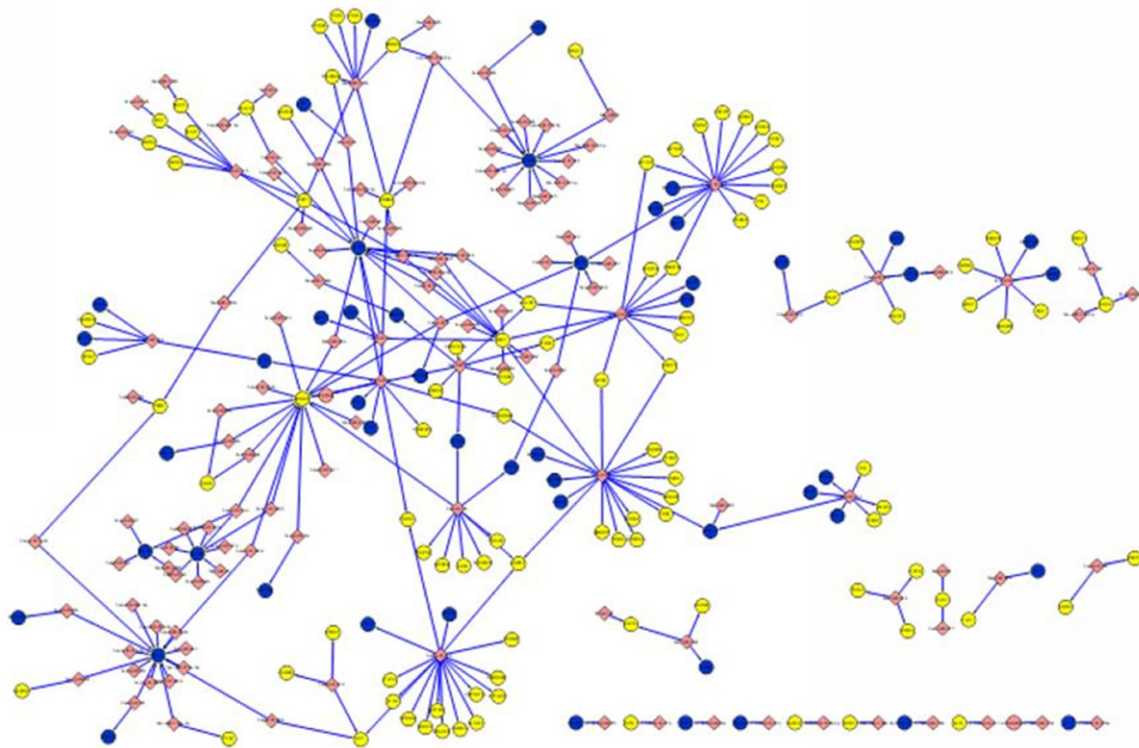


Figure 1. The constructed miRNA-target regulatory network. Pink diamonds represent miRNAs, yellow circles represent the up-regulated target DEGs and blue circles represent down-regulated target DEGs.

including defense response (FDR = 6.58E-07), inflammatory response (FDR = 7.54E-07), response to wounding (FDR = 6.14E-06), cation homeostasis (FDR = 0.02514) and cytokine-mediated signaling pathway (FDR = 0.031872) were identified for down-regulated DEGs.

Construction of miRNA-target regulatory network

According to the experimentally validated regulatory relationships between DEGs and miRNAs, the miRNA-target regulatory network containing 280 nodes and 286 edges were con-

structed (**Figure 1**). Most target genes of some miRNAs, such as hsa-miR-155, hsa-miR-373, hsa-miR-1, hsa-miR-30a, hsa-miR-124, hsa-miR-125b, hsa-miR-192 and hsa-let-7b, were differentially expressed in pancreatic cancer samples. Furthermore, the miRNAs were respectively enriched for up- and down-regulated DEGs with the cut-off criterion of $P < 0.05$ (**Table 4**). Total 4 miRNAs including hsa-miR-145 ($P = 0.0113$), hsa-miR-191 ($P = 0.0115$), hsa-miR-125b ($P = 0.0351$) and hsa-miR-124 ($P = 0.0439$) were selected for the up-regulated DEGs, while 1 miRNA hsa-miR-185 ($P = 0.0271$) was enriched for down-regulated

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Table 4. The miRNAs for identified up- and down-regulated DEGs

miRNA	Up-regulated target genes	Down-regulated target genes	P-value
hsa-miR-145	IRS1, KRT7, MUC1, MYO6, STAT1, TMOD3	MYC	0.0113
hsa-miR-191	SOX4, TMC7	0	0.0115
hsa-miR-125b	CGN, ERBB2, ERBB3, KCNS3, KRT7, NKIRAS2, VDR	QSOX2	0.0351
hsa-miR-124	AP1M2, CTNND1, ELOVL1, F11R, FAM83H, HTATIP2, LRRC1, PLOD3, PPP1R13L, PTTG1P, SP1, TJP2, TNFRSF21, TRIM29, UHRF1	CCL2, TWIST2	0.0439
hsa-miR-185	CORO2B, NTRK3	0	0.0271

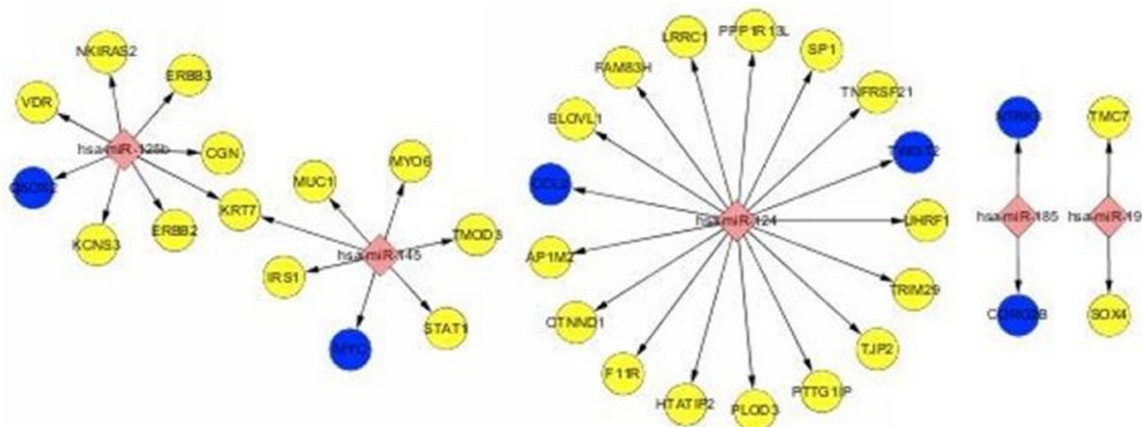


Figure 2. Regulatory relations of significantly enriched miRNAs with their corresponding up- and down-regulated DEGs. Pink diamonds represent miRNAs, yellow circles represent the up-regulated target DEGs and blue circles represent down-regulated target DEGs.

DEGs (NTRK3 and CORO2B). What's more, the regulatory relationships of significantly enriched miRNAs and their corresponding up- and down-regulated target DEGs were shown in **Figure 2**.

Discussion

Pancreatic cancer is a fatal cancer with an extremely poor 5-year survival of only about 4% and a majority of patient with advanced stage have a median survival time of less than one year [28, 29]. Therefore, new approaches for early detection and treatment of pancreatic cancer are urgently needed. In this present study, the downloaded gene expression profile of human pancreatic cancer samples and non-malignant pancreas samples were investigated using bioinformatics methods.

Our results showed that 1113 DEGs were obtained, including 697 (62.62%) up-regulated genes and 416 (37.38%) down-regulated genes. The up- and down-regulated DEGs were significantly associated with the adhesion and immune response, respectively. Donahue et al. have found that survival-correlated genes involved in

pancreatic cancer are enriched in ERBB signaling, focal adhesion, insulin signaling, and MAPK pathways [30]. Furthermore, KRAS mutation could activate Hedgehog signaling and inflammatory pathways, presenting in over 90% of pancreatic cancers [31, 32]. Therefore, the identified DEGs might have the potential to be used as biomarkers for pancreatic cancers to some extent.

Furthermore, 4 miRNAs including hsa-miR-145, hsa-miR-191, hsa-miR-125b and hsa-miR-124 were enriched for the up-regulated DEGs. It has been reported that miR-221, -100, -125b and -21 are significantly increased in pancreatic adenocarcinoma and endocrine pancreas cancer [33]. From the constructed miRNA-target regulatory network, we could find that ERBB2 and ERBB3 were up-regulated by hsa-miR-125b. What's more, cytokeratins 7 (KRT7) could be simultaneously up-regulated by hsa-miR-145 and hsa-miR-125b. Detection of CRTK 7 and 20 expression is widely used for distinguishing adenocarcinomas from different sites, and the extrahepatic biliary tract and pancreas carcinomas are strongly positive for

CRK7 [34, 35]. Meanwhile, STAT1 was also up-regulated by hsa-miR-145. Wu et al. have found that activation of the STAT1 pathway may play an important role in the increased reactive oxygen species production which could contribute to a pro-inflammatory milieu in the pancreas and the pathophysiologic characteristics of human pancreatic cancer [36]. We also found that specificity protein 1 (SP1) was up-regulated by hsa-miR-124 according to the constructed miRNA-target regulatory network. Wang et al. have reported that miR-124 including miR-124-1, miR-124-2 and miR-124-3 which could inhibited cell proliferation, invasion and metastasis are highly methylated in pancreatic cancer tissues compared with in non-cancerous tissues [37]. It has been proved that SP1 knockdown significantly inhibited pancreatic cancer angiogenesis, growth, and metastasis mouse model and SP1 inhibitor mithramycin A could suppress the expression of SP1 and its downstream targets of VEGF, platelet-derived growth factor (PDGF), and epidermal growth factor receptor (EGFR) in growing tumors [38].

Additionally, 1 miRNA hsa-miR-185 was enriched for down-regulated DEGs (NTRK3 and CORO2B) in our study. The expression level of miR-185 has been detected to be significantly up-regulated in pancreatic cancer samples [39]. NTRK3 is a member of the neurotrophin receptor family regulating cell survival and somatic mutation in NTRK3 has been identified in pancreatic cancer [40, 41]. However, few study has investigated the relation between the CORO2B and pancreatic cancer. We speculate that miR-185 might participate in the development of pancreatic cancer by down-regulating the expression levels of NTRK3 and CORO2B.

Conclusions

In conclusion, the identified DEGs and enriched miRNAs regulating up- and down-regulated DEGs might play important roles in the development of pancreatic cancer. Therefore, these selected gene and microRNA biomarkers have the potential to be used for diagnosis and treatment of pancreatic cancer. However, these results still need to be further confirmed by experimental study with larger sample size.

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Disclosure of conflict of interest

None.

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