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Identification of miRNA-mRNA crosstalk in pancreatic cancer by integrating transcriptome analysis

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Abstract. – OBJECTIVE: Pancreatic cancer is one of the most lethal diseases, and the pathogenesis remains largely unknown. To this end, we performed an integrated analysis of miRNA and mRNA expression data to explore the deregulation of miRNA and mRNA and regulatory processes underlying pancreatic cancer.

MATERIALS AND METHODS: We combined mRNA and miRNA expression data with miRNA target predictions to infer new miRNA regulation activities in pancreatic cancer. We first integrated miRNA and mRNA expression profiling separately to identify differently expressed miRNA and mRNA in pancreatic cancer. Then we adopted miRWalk databases prediction to obtain potential target genes of differently expressed miRNA, and compared these target genes to the gene list of integrated mRNA expression profiling to select differentially expressed miRNA-target gene whose expression was reversely correlated with that of corresponding miRNAs. Gene Ontology (GO) classification analyses and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were employed to understand the functions and pathways of miRNA target genes. Finally we construct a miRNA-target gene regulatory network.

RESULTS: 42 differentially expressed miRNAs, 1376 differentially expressed mRNAs were identified by combining three expression profiles of miRNA and mRNA separately in pancreatic cancer, 146 miRNA target genes were found in the gene list of integrated mRNA expression profiling based on bioinformatics prediction. Functional annotation was performed to understand the functions and pathways of miRNA target genes. Finally, we constructed a miRNA-target gene regulatory network including 206 miRNA-target gene pairs. Five miRNAs (hsa-miR-130b, hsa-miR-106b, hsa-miR-181c, hsa-miR-153 and hsa-miR-125a-5p) demonstrated the highest connectivities, whereas three miRNAs (MYC, E2F1 and IL6) were the mRNAs with the highest connectivities.

CONCLUSIONS: Our findings may provide new insights into the knowledge of molecular mechanisms of pancreatic cancer and development of novel targeting therapies.

Key Words:
Integrated analysis, mRNA expression data, miRNA expression data, Pancreatic cancer, miRNA target genes.

Introduction

Pancreatic cancer remains one of the most aggressive malignancies characterized by an extremely low 5-year survival rate[1,2]. Due to its aggressive nature, most of patients are diagnosed in advanced stages, which limits the potential for therapeutic intervention by the time of diagnosis and leads to a poor prognosis[3]. In view of currently few therapeutic options for patients with pancreatic cancer, and it is urgently needed to discover new insights into the pathogenesis of this lethal disease.

MiRNAs are small (~22 nucleotides) non-coding RNAs with gene regulatory functions by binding to the 3'-untranslated region (3'-UTR) of their target mRNA resulting in either translational repression or mRNA degradation[4]. It has been reported that miRNAs have distinct expression profiles in various human diseases, particularly cancers[5,6]. miRNAs may be involved in various human cancers as cancer suppressors or oncogenes.

Advances in molecular biology have increased the understanding of the pathophysiology of pancreatic cancer. Various genome-wide mRNA and miRNA expression profiling studies using mi-

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croarray-based approaches have provided important insights into the phenotypic characteristics of pancreatic cancer[7-10]. Amounts of genes have been identified to be associated with pancreatic cancer such as the K-ras, p53, p16, and Smad4 genes, leading to the behavior of this aggressive malignancy[11].

Many miRNAs have been reported to alter pancreatic cancer proliferation and/or migration *in vitro* and *in vivo*. The expression of miR-132 in cell lines derived from PDAC promoted cell proliferation and inhibited colony formation[12]. miR-96 regulated pancreatic cancer cell proliferation, invasion, apoptosis to play a role in tumor growth by targeting KRAS13. miR-198 was implicated in tumor growth, metastasis and survival through direct targeting MSLN, PBX-1, and VCP as a central tumor suppressor in pancreatic cancer[14]. miR-197 was reported recently to function in the epithelial-mesenchymal transition in pancreatic cancer cells by targeting p120 catenin[15].

In this study, we integrated multiple expression profiles of mRNAs and miRNAs to construct a novel miRNA-mRNA regulatory network in pancreatic cancer. Our data may provide an important contribution to future investigations aimed at elucidating the mechanisms of pancreatic cancer.

Materials and Methods

Gene Expression Profiles

We searched the Gene Expression Omnibus database (GEO, http://www.ncbi.nlm.nih.gov/geo) for mRNA and miRNA expression profiling studies in pancreatic cancer. GEO served as a public repository for gene expression datasets, initiated by the growing demand for a public repository for high-throughput gene expression data[16]. We only retained the microarray studies between tumor and normal tissues, and miRNA and mRNA expression profilings were both performed in one study to minimize the heterogeneity of our analysis. The following information was extracted from each identified study: GEO accession number, platform, number of cases and controls, country, time and author.

Differential Expression of miRNA and mRNA

The heterogeneity among different microarray datasets caused by different platforms, different gene nomenclature and different clinical simple, makes it difficult to compare the microarray datasets directly. Consequently, we preprocessed the raw microarray data of each study by Quantile normalization and log2 transformation to obtain intensity values. The MATrix LABoratory (MATLAB) software was used to identify the differently expressed probe sets in the tumor tissues compared to the normal tissues by two-tailed Student's t-test, then P-value and effect size of individual microarray study were calculated. Fisher's method was used to combine *p*-value from multiple studies, and the random effects model was used to combine effect size from multiple studies. We selected differently expressed mRNA with criterion of p-value < 0.01 and effect size > 1.5, while for differently expressed miRNA with criterion of p-value < 0.05 and effect size > 1.0.

Identification of Differently Expressed miRNA Target Genes

As miRNAs play their role by regulating the expression of target proteins, precise miRNA target prediction is important for the research of miRNA function. We predicted putative targets of differentially expressed miRNAs by six bioinformatic algorithms (DIANAmT, miRanda, miRDB, miRWalk, PICTAR and Targetscan), and furthermore searched miRNA target genes with experiment validation by using miRWalk databases (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/)[17], and the targets recorded by ≥ 4 algorithms or validated by experiments were selected to compare with the gene list of integrated mRNA expression profiling. As miRNAs tend to down-regulate the expression of their target genes, we selected differentially expressed target genes whose expression was reversely correlated with that of corresponding miRNAs, to subject to further investigation[18-22].

Functional Annotation

To gain insights into the biological functions of these miRNA target genes, we performed the Gene Ontology (GO) classification. To detect the potential pathway of miRNA target genes, we also performed the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. KEGG pathway database is a recognized and comprehensive database including all kinds of biochemistry pathways[23]. The online based software GENECODIS was utilized in those functional annotation[24].

Constructing Regulatory Network Between miRNAs and Their Targets

The posttranscriptional regulatory network is defined as a directed and bipartite graph in which expressions of miRNA-target gene interacting pairs are reversely correlated. Based on the identified miRNA-target gene interacting pairs, we conducted a regulatory network between miRNAs and genes in pancreatic cancer and visualized with Cytoscape[25].

Results

Differentially Expressed miRNAs and mRNAs in the Pancreatic Cancer

In this work, we collected a total of 3 expression profiling studies according to the inclusion criteria, and it contained 60 samples of pancreatic cancer and 21 samples of normal control, respectively (Table I). After normalization of the original miRNA and mRNA expression data, we performed differentially expressed analysis between pancreatic cancer and normal control samples using MATLAB. Finally, 42 miRNAs were regarded as significantly differentially expressed under the threshold of p-value < 0.05 and effect size > 1.0, with 24 up-regulated and 18 down-regulated miRNAs (Table II). The up-regulated miRNA with the lowest p-value was hsa-miR-106b, which was found to be implicated in several types of cancers such as gastric cancer[26], breast cancer[27], endometrial cancer[28] by targeting PTEN, TWIST1, but hasn't been identified in pancreatic cancer yet. The down-regulated gene with the lowest p-value was hsa-miR-216a, which was detected at lower concentrations in feces of pancreatic cancer patients when compared to controls[29].

A total of 1376 genes were identified to be differentially expressed in pancreatic cancer under the threshold of p-value < 0.01 and effect size > 1.5, including 671 up-regulated and 705 down-regulated genes. The full list of these genes was provided as Supplementary Table I.

Identification of Differently Expressed miRNA Target Genes

We combined mRNA and miRNA expression data with miRNA target predictions to obtain genuine miRNA targets. As a result, 24 miRNAs and 146 genes formed 206 miRNA-target gene pairs with an inverse correlation of expression. 154 miRNA-target gene pairs were identified for the up-regulated miRNA, of which 70 miRNA-target gene pairs were validated by experiments. 52 miRNA-target gene pairs were identified for the down-regulated miRNA with 11 validated miRNA-target gene pairs (Table III). The target prediction of 18 miRNAs isn't available in miRWalk databases.

GO Classification and KEGG Pathways of miRNA Target Genes

To gain insights into the biological roles of differently expressed miRNA target genes, we performed the GO classification enrichment analysis. Genes that showed a nominal significance level of p < 0.01 were selected and were tested against the background set of all genes with GO annotations.

Table I. Characteristics of mRNA and miRNA expression profiling of the pancreatic cancers.

GEO ID	Platform	Samples (case: control)	Country	Time	Author
GSE43797	GPL10558 Illumina HumanHT-12 V4.0 expression beadchip GPL15159 Agilent-031181 Unrestricted_Human_miRNA_V16.0_Microarray 030840 (Probe Name version)	26:5	Korea	2014	Park M
GSE41372	GPL6244 [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version] GPL16142 NanoString nCounter Human miRNA assay (v1)	9:9	Italy	2014	Frampton AE
GSE32688	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array GPL7723 miRCURY LNA microRNA Array, v.11.0 - hsa, mmu & rno	25:7	USA	2012	Donahue TR

Table II. List of differentially expressed miRNAs.

MiRNAs	*p* value	Effect size
Up-regulated miRNAs		
hsa-miR-106b	0.0013968	-1.3456
hsa-miR-498	0.002329	-1.099
hsa-miR-210	0.0025023	-1.4964
hsa-miR-135b	0.0041736	-1.4552
hsa-miR-331-3p	0.0041877	-1.3291
hsa-miR-183	0.0052555	-1.097
hsa-miR-425	0.0059097	-1.4193
hsa-miR-125a-5p	0.0074092	-1.2296
hsa-miR-99b	0.0080654	-1.1336
hsa-miR-135a	0.0087537	-1.0829
hsa-miR-24	0.010172	-1.1558
hsa-miR-93	0.010462	-1.1824
hsa-miR-484	0.013076	-1.2186
hsa-miR-23a	0.014026	-1.1386
hsa-miR-491-5p	0.014287	-1.0703
hsa-miR-128	0.015878	-1.0149
hsa-miR-149	0.017938	-1.1139
hsa-miR-345	0.018929	-1.0863
hsa-miR-181c	0.022311	-1.1816
hsa-miR-320a	0.024712	-1.0042
hsa-miR-153	0.0272	-1.0247
hsa-miR-501-3p	0.0274	-1.123
hsa-miR-197	0.028031	-1.103
hsa-miR-222	0.043387	-1.0561
Down-regulated miRNAs		
hsa-miR-216a	4.81E-06	2.1013
hsa-miR-217	1.00E-05	1.8926
hsa-miR-564	0.001543	1.4442
hsa-miR-130b	0.0027725	1.2476
hsa-miR-299-3p	0.0034827	1.0907
hsa-miR-1179	0.0039804	1.1227
hsa-miR-944	0.0069999	1.2026
hsa-miR-381	0.009707	1.0589
hsa-miR-553	0.011603	1.0607
hsa-miR-1275	0.012952	1.1411
hsa-miR-551a	0.014108	1.113
hsa-miR-548l	0.016856	1.0308
hsa-miR-125a-3p	0.016867	1.0684
hsa-miR-557	0.018052	1.0404
hsa-miR-513a-5p	0.019829	1.0459
hsa-miR-1262	0.022754	1.099
hsa-miR-1302	0.027825	1.0485
hsa-miR-520a-5p	0.042534	1.0713

We found that negative regulation of transcription from RNA polymerase II promoter (GO: 0000122, p = 7.19E-07) and signal transduction (GO: 0007165, p = 5.14E-06) were significantly enriched for biological processes. While for molecular functions were protein binding (GO: 0005515, p = 2.84E-14) and metal ion binding (GO: 0046872, p = 5.90E-11), and for cellular component were cytoplasm (GO: 0005737, p = 5.15E-22) and plasma membrane (GO: 0005886, p = 5.34E-18) (Table IV, Figure 1A).

We also performed the KEGG pathway enrichment analysis for differently expressed miRNA target genes. Hypergeometric test with p value < 0.05 were used as the criteria for pathway detection. The most significant pathway in our analysis was pathways in cancer (p = 4.92E-06). Furthermore, focal adhesion (p = 1.82e-04) and melanogenesis (p = 5.19E-04) are also highly enriched (Table V, Figure 1B).

The Regulatory Network of miRNAs and Target Genes

The miRNA-target genes regulatory network was constructed with the miRNA-target gene pairs by Cytoscape software. Using the 206 miRNA-target gene pairs, a miRNA-target gene regulatory network was constructed (Figure 2). In this network, hsa-miR-130b, hsa-miR-106b, hsa-miR-181c, hsa-miR-153 and hsa-miR-125a-5p, which regulate 39, 24, 23, 13, and 12 targets, respectively, demonstrated the highest connectivities, whereas MYC, E2F1, IL6, BBC3 and RRBP1, which were regulated by 9, 7, 5, 5 and 4 miRNAs, respectively, were the mRNAs with the highest connectivities.

Discussion

In this study, we combined mRNA and miRNA transcriptomes of pancreatic cancer with miRNA target predictions to detect not only the expression dynamics of mRNA and miRNA, but also the interplay of genes and miRNAs during the tumorigenic processes. In total, 42 miRNAs were found to be differentially expressed in the pancreatic cancer by utilizing the acquired data sets. Importantly, most of these miRNAs identified in our work have been reported previously to be involved in the development of pancreatic cancer and other cancers. miR-210 are implicated in regulating the hypoxic response of tumor cells and tumor growth by targeting E2F3, EFNA3, GIT2, MNT, ZNF462 and EGR3 in pancreatic cancer[30,31]. miR-183 was recently reported to be dysregulated, and regulate cell proliferation by downregulation of Bmi-1 expression in pancreatic cancer[32]. miR-197 was identified to be up-regulated in pancreatic cancer cells to induce the EMT process by targeting p120 catenin[15]. miR-491-5p displayed a significantly low level of expression in pancreatic cancer cell and mediated cell apoptosis by targeting both Bcl-xL and TP53[33].

Table III. The 146 miRNA target genes anticorrelated with the expressions of 24 differentially expression miRNAs.

miRNA	Regulation (miRNAs)	Count of targets	Target mRNAs
hsa-miR-106b	Up	24	ACADSB, BBC3, E2F1, FAM129A, FAM46C, IL6, INTS6, LMO3, MKNK2, MUM1L1, MYC, NR4A2, NR4A3, ORMDL3, OSR1, PCAF, PEX5L, PKHD1, PPP6C, RNF125, SERP1, TRIP11, TRPV6, VLDLR
hsa-miR-125a-5p	Up	12	E2F1, ENPP1, EPO, IL22RA1, LIFR, MYC, PCAF, PPIF, RRBP1, SSTR3, SYVN1, UBE2R2
hsa-miR-128	Up	1	PDCD4
hsa-miR-135a	Up	10	ADARB2, DLK1, MED13, MGAT4A, PDE8B, PELI2, PRDM16, RRBP1, TMEM97, VLDLR,
hsa-miR-135b	Up	6	ADARB2, MGAT4A, PDE8B, PELI2, TMEM97, VLDLR
hsa-miR-149	Up	8	E2F1, EPHB3, MYC, PCAF, PSAT1, SAV1, SLC4A4, SUSD5
hsa-miR-153	Up	13	CCDC110, ERO1LB, GATM, GDF10, ITSN2, MTMR12, PCMTD1, PRDM16, RPL22, RYR2, SGK3, SLC4A4, TXNDC11
hsa-miR-181c	Up	23	AKAP7, ENPP1, F11, FUT9, GATM, HCN1, ID4, IL6, LMAN1, LMO3, MTMR12, MYC, NR4A3, NSUN7, PKNOX2, SEL1L, SGK3, SLC19A2, SLC25A25, SYT15, TBC1D4, TIFA, TMEM181
hsa-miR-183	Up	11	ABCA8, AMD1, EML4, GFI1, MON2, MSH6, PDCD4, PRPH2, RET, SEL1L, SLC1A2
hsa-miR-197	Up	2	IL6, MYC
hsa-miR-210	Up	6	CTRL, CXCL12, MYC, OSR1, P4HB, PSAT1
hsa-miR-222	Up	10	BBC3, BMX, DPT, EGF, FOXO3, MYC, PDCD4, PSAT1, RET, RRBP1
hsa-miR-23a	Up	5	CXCL12, DLL1, E2F1, FOXO3, MYC
hsa-miR-24	Up	3	FAF1, FOXO3, MYC
hsa-miR-320a	Up	3	AQP1, BBC3, E2F1
hsa-miR-331-3p	Up	2	BBC3, E2F1
hsa-miR-491-5p	Up	4	CCKBR, FGFR1, MAPK9, NIT1
hsa-miR-498	Up	1	FAF1
hsa-miR-93	Up	7	BBC3, E2F1, FGFR1, IL6, MAPK9, NIT1, PCAF
hsa-miR-99b	Up	3	IL6, PSAT1, RRBP1
hsa-miR-125a-3p	Down	10	C12orf5, CXCR4, E2F3, EFR3A, GPRC5A, KPNA2, MAL2, RB1, THUMPD3, TSC22D1
hsa-miR-130b	Down	39	ACVR1, ARFIP1, ARHGAP1, ATP6V0E1, B4GALT5, BMPR2, BTBD10, CALM2, CBFB, CNOT6, CXCR4, DCP2, EFNB2, ERBB2IP, GMFB, HOXB3, ITGB1, KIAA1217, KLHL20, LRP8, MASTL, MAT2B, MYO1D, NME7, PGM2L1, RALBP1, RASA1, RNF145, RPGRIP1L, RUNX2, SGCB, SNAPIN, SNX27, SOX4, SPOCK1, TMEM55A, TRPS1, UBA3, YWHAB
hsa-miR-216a	Down	1	ELOVL6
hsa-miR-299-3p	Down	2	ITGA2, ITGA3

miRNAs perform their regulatory function by degrading or inhibiting the translation of its target genes. Consequently it is of vital importance to identify miRNA target genes to understand the biological functions of miRNAs. In this study we integrated mRNA and miRNA expression data with miRNA target predictions by the miRWalk database to discover novel regulatory relation between miRNAs and mRNAs. As a result, 24 miRNAs and 146 genes formed 206 miRNA-target gene pairs with an inverse correlation of expression. The target prediction of 18 miRNAs isn't available in miRWalk databases.

Functional annotation of the 146 miRNA target genes was performed to estimate the biological roles of differentially expressed miRNAs in pancreatic cancer. We found that the enriched GO term of the target genes for biological processes was negative regulation of apoptotic process, positive regulation of transcription, DNA-dependent and positive regulation of cell proliferation, which are totally according to the biologic behavior of cancer cell. KEGG pathway enrichment analysis showed that Pathways in cancer was statistically enriched containing many genes including E2F3, E2F1,

Table IV. GO functional annotation of differentially expression miRNA target genes (Top 15).

GO ID	GO Term	Count	%	FDR
Biological process				
GO:0043066	Negative regulation of apoptotic process	11	7.53E-02	1.28E-05
GO:0045893	Positive regulation of transcription, DNA-dependent	14	9.59E-02	1.42E-05
GO:0008284	Positive regulation of cell proliferation	12	8.22E-02	1.69E-05
GO:0007165	Signal transduction	19	1.30E-01	1.59E-04
GO:0045669	Positive regulation of osteoblast differentiation	5	3.42E-02	1.82E-04
GO:0042551	Neuron maturation	3	2.05E-02	6.44E-04
GO:0033554	Cellular response to stress	3	2.05E-02	8.26E-04
GO:0051726	Regulation of cell cycle	5	3.42E-02	9.50E-04
GO:0045892	Negative regulation of transcription, DNA-dependent	10	6.85E-02	9.64E-04
GO:0000085	G2 phase of mitotic cell cycle	3	2.05E-02	1.03E-03
GO:0071456	Cellular response to hypoxia	4	2.74E-02	1.24E-03
GO:0045727	Positive regulation of translation	4	2.74E-02	1.26E-03
GO:0071930	Negative regulation of transcription involved in G1/S phase of mitotic cell cycle	2	1.37E-02	1.28E-03
GO:0072239	Metanephric glomerulus vasculature development	2	1.37E-02	1.28E-03
GO:0007155	Cell adhesion	11	7.53E-02	1.77E-03
Cellular component				
GO:0005737	Cytoplasm	54	3.70E-01	3.07E-08
GO:0016020	Membrane	45	3.08E-01	7.27E-08
GO:0016021	Integral to membrane	43	2.95E-01	5.29E-06
GO:0005829	Cytosol	27	1.85E-01	1.57E-05
GO:0005634	Nucleus	47	3.22E-01	2.16E-05
GO:0005886	Plasma membrane	36	2.47E-01	2.43E-05
GO:0005615	Extracellular space	13	8.90E-02	6.97E-04
GO:0005887	Integral to plasma membrane	15	1.03E-01	7.46E-04
GO:0009986	Cell surface	8	5.48E-02	8.06E-04
GO:0035189	Rb-E2F complex	2	1.37E-02	1.78E-03
GO:0009925	Basal plasma membrane	3	2.05E-02	2.45E-03
GO:0008305	Integrin complex	3	2.05E-02	3.53E-03
GO:0030056	Hemidesmosome	2	1.37E-02	6.32E-03
GO:0017053	Transcriptional repressor complex	3	2.05E-02	7.90E-03
GO:0030673	Axolemma	2	1.37E-02	1.07E-02
Molecular function				
GO:0005515	Protein binding	65	4.45E-01	2.70E-18
GO:0004872	Receptor activity	21	1.44E-01	4.99E-04
GO:0001047	Core promoter binding	3	2.05E-02	2.02E-03
GO:0003700	Sequence-specific DNA binding transcription factor activity factor activity	14	9.59E-02	2.47E-03
GO:0001948	Glycoprotein binding	4	2.74E-02	2.60E-03
GO:0005524	ATP binding	18	1.23E-01	3.45E-03
GO:0030229	Very-low-density lipoprotein particle receptor activity	2	1.37E-02	4.07E-03
GO:0034187	Apolipoprotein E binding	2	1.37E-02	4.07E-03
GO:0034237	Protein kinase A regulatory subunit binding	2	1.37E-02	1.26E-02
GO:0019904	Protein domain specific binding	5	3.42E-02	1.57E-02
GO:0005509	Calcium ion binding	10	6.85E-02	1.59E-02
GO:0008083	Growth factor activity	5	3.42E-02	1.61E-02
GO:0048306	Calcium-dependent protein binding	3	2.05E-02	1.61E-02
GO:0004675	Transmembrane receptor protein serine/ threonine kinase activity	2	1.37E-02	2.09E-02
GO:0046872	Metal ion binding	24	1.64E-01	2.33E-02

RALBP1, MAPK9, EGF and RB1, some of which are also implicated in pancreatic cancer. These genes functioned as oncogenes and tumor suppressor genes or were involved in the regulation of oncogenes and tumor suppressor genes to trigger the tumorigenesis of pancreatic cancer.

In addition a miRNA-target gene regulatory network was constructed with miRNA-target gene pairs. In this network, hsa-miR-130b

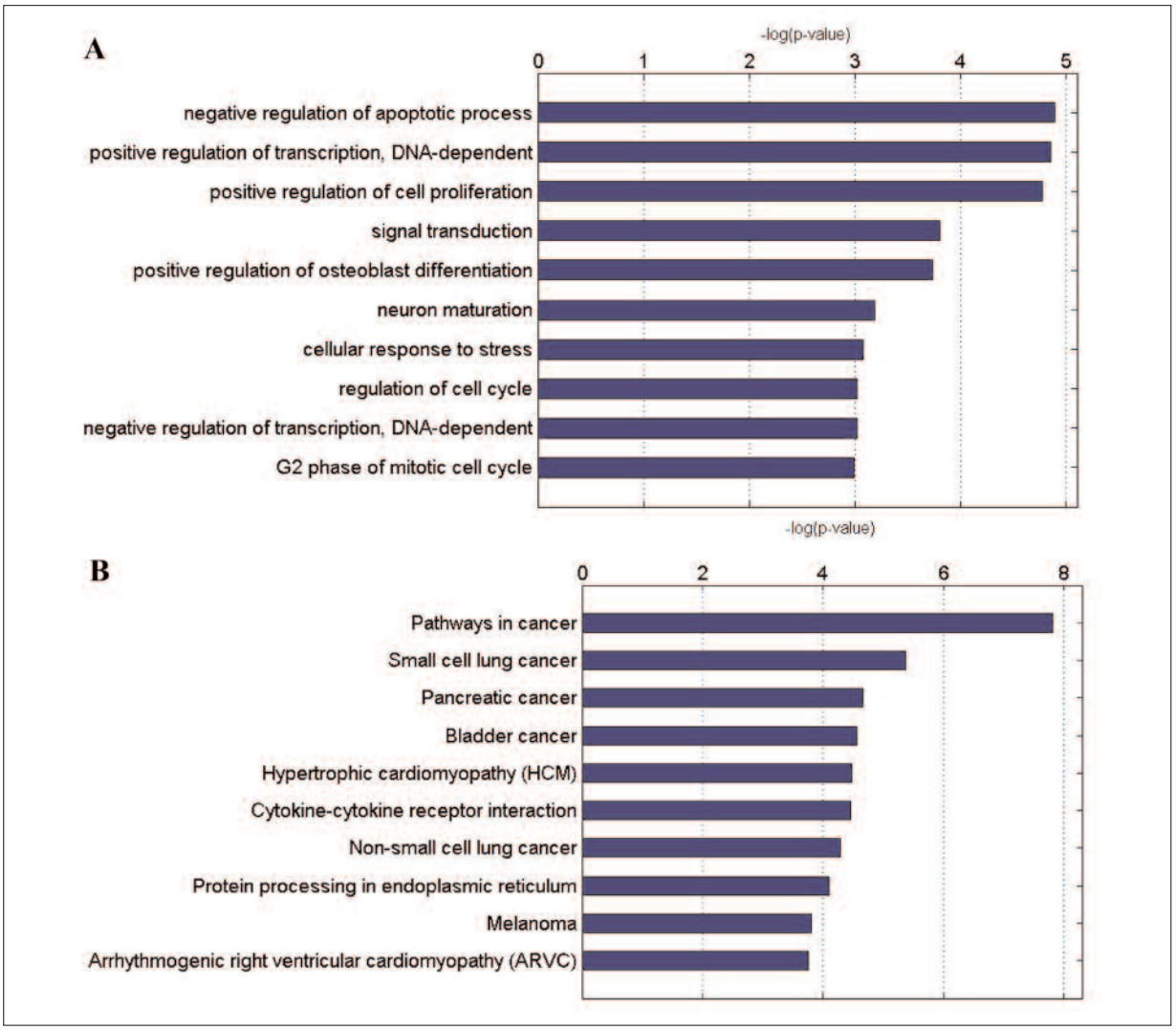

Figure 1. The significantly enriched functional annotation of differentially expressed miRNA target genes. ***A,*** The top 10 enriched GO categories for biological process. ***B,*** The top 10 enriched KEGG pathway.

demonstrated to be the miRNA with the highest connectivities, whereas MYC was the mRNA with the highest connectivities suggesting that hsa-miR-130b and MYC may play important roles in the tumorigenesis of pancreatic cancer. To data, miR-130b is significantly deregulated in various human tumor types such as gastric cancer[34], glioma[35], renal cell cancer[36] and endometrial cancer[37]. Recently, a study[38] found that miR-130b was significantly down-regulated in 52 pairs of pancreatic cancer tissues and five cell lines, and miR-130b inhibited cell proliferation and invasion in pancreatic cancer by targeting STAT3. MYC regulated by 9 miRNAs, is critical not only for the proliferation and development of normal pancreas but also for pancreatic cancer, was verified by experimental data in human and animals to be a key oncogene in pancreatic cancer[39-42].

Conclusions

In summary, we identified 42 differentially expressed miRNAs, 1376 differentially expressed mRNAs, and 146 miRNA target genes whose expression was reversely correlated with that of corresponding miRNAs in pancreatic cancer, and constructed a regulatory network including 206 miRNA-target gene pairs. We also found that five miRNAs (hsa-miR-130b, hsa-miR-106b, hsa-miR-181c, hsa-miR-153 and hsa-miR-125a-5p)

Table V. KEGG pathway enrichment analysis of differentially expression miRNA target genes (Top 15).

KEGG ID	KEGG term	Count	FDR	Genes
hsa05200	Pathways in cancer	14	1.55E-08	IL6, E2F3, ITGA3, E2F1, ITGA2, MSH6, RALBP1, MAPK9, FGFR1, EGF, MYC, ITGB1, RET, RB1
hsa05222	Small cell lung cancer	7	4.30E-06	E2F3, ITGA3, E2F1, ITGA2, MYC, ITGB1, RB1
hsa05212	Pancreatic cancer	6	2.13E-05	E2F3, E2F1, RALBP1, MAPK9, EGF, RB1
hsa05219	Bladder cancer	5	2.77E-05	E2F3, E2F1, EGF, MYC, RB1
hsa05410	Hypertrophic cardiomyopathy (HCM)	6	3.27E-05	IL6, RYR2, ITGA3, ITGA2, SGCB, ITGB1
hsa04060	Cytokine-cytokine receptor interaction	9	3.48E-05	IL6, CXCR4, EPO, LIFR, BMPR2, ACVR1, EGF, CXCL12, IL22RA1
hsa05223	Non-small cell lung cancer	5	5.15E-05	E2F3, E2F1, EGF, RB1, FOXO3
hsa04141	Protein processing in endoplasmic reticulum	7	8.11E-05	P4HB, ERO1LB, MAPK9, SYVN1, RRBP1, SEL1L, LMAN1
hsa05218	Melanoma	5	0.0001593	E2F3, E2F1, FGFR1, EGF, RB1
hsa05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	5	0.0001761	RYR2, ITGA3, ITGA2, SGCB, ITGB1
hsa04360	Axon guidance	6	0.0001962	CXCR4, ITGB1, EPHB3, RASA1, CXCL12, EFNB2
hsa05414	Dilated cardiomyopathy	5	0.0003544	RYR2, ITGA3, ITGA2, SGCB, ITGB1
hsa05215	Prostate cancer	5	0.0003636	E2F3, E2F1, FGFR1, EGF, RB1
hsa05214	Glioma	4	0.0012477	E2F3, E2F1, EGF, RB1
hsa04110	Cell cycle	5	0.0014235	E2F3, E2F1, MYC, YWHAB, RB1

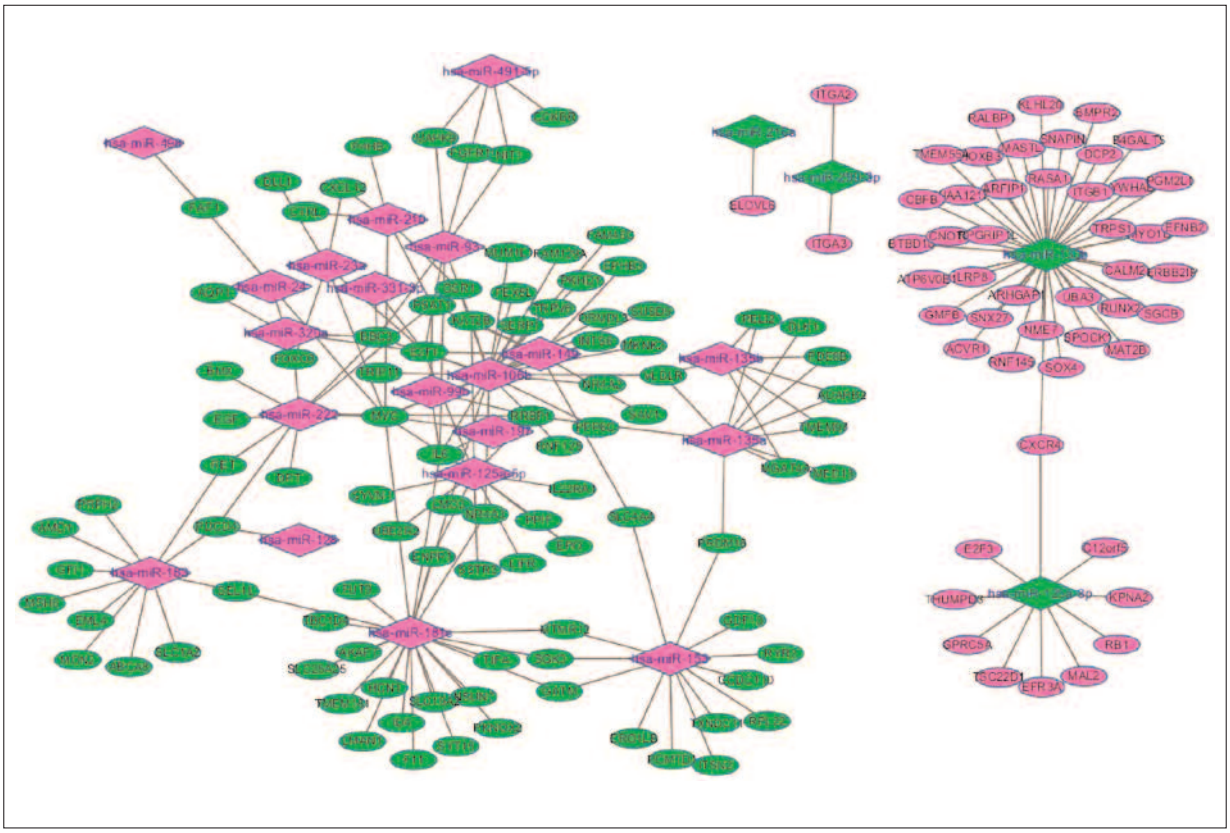

Figure 2. The regulatory network between miRNAs and target genes in pancreatic cancer. The diamonds and ellipses represent the miRNAs and genes, respectively. The red and green colors represent the relatively high and low expression, respectively. The larger geometric drawing indicates the more miRNAs or genes interacted with it.

demonstrated the highest connectivities, whereas five miRNAs (MYC, E2F1, IL6, BBC3 and RRBP1) were the mRNAs with the highest connectivities. More especially, hsa-miR-130b and MYC was highly correlated with tumorigenesis of pancreatic cancer. Our data add some new insights into the molecular mechanism of tumorigenesis of pancreatic cancer, and may be helpful for the successful identification of therapeutic targets for pancreatic cancer and the development of effective targeted therapies.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Raimondi S, Maisonneuve P, Lowenfels AB. Epidemiology of pancreatic cancer: an overview. Nature reviews. Gastroenterol Hepatol 2009; 6: 699-708.
2) Li D, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. Lancet 2004; 363: 1049-1057.
3) Pliarchopoulou K, Pectasides D. Pancreatic cancer: current and future treatment strategies. Cancer Treat Rev 2009; 35: 431-436.
4) Winter J, Diederichs S. MicroRNA biogenesis and cancer. Methods Mol Biol 2011; 676: 3-22.
5) Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 2006; 103: 2257-2261.
6) Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. Nature 2005; 435: 834-838.
7) Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 2008; 321: 1801-1806.
8) Takikawa T, Masamune A, Hamada S, Nakano E, Yoshida N, Shimosegawa T. miR-210 regulates the interaction between pancreatic cancer cells and stellate cells. Biochem Biophys Res Commun 2013; 437: 433-439.
9) He H, Di Y, Liang M, Yang F, Yao L, Hao S, Li J, Jiang Y, Jin C, Fu D. The microRNA-218 and ROBO-1 signaling axis correlates with the lymphatic metastasis of pancreatic cancer. Oncol Rep 2013; 30: 651-658.
10) Campbell PJ, Yachida S, Mudie LJ, Stephens PJ, Pleasance ED, Stebbings LA, Morsberger LA, Latimer C, McLaren S, Lin ML, McBride DJ, Varela I, Nik-Zainal SA, Leroy C, Jia M, Menzies A, Butler AP, Teague JW, Griffin CA, Burton J, Swerdlow H, Quail MA, Stratton MR, Iacobuzio-Donahue C, Futreal PAL. The patterns and dynamics of genomic instability in metastatic pancreatic cancer. Nature 2010; 467: 1109-1113.
11) Welsch T, Kleeff J, Friess H. Molecular pathogenesis of pancreatic cancer: advances and challenges. Curr Mol Med 2007; 7: 504-521.
12) Zhang S, Hao J, Xie F, Hu X, Liu C, Tong J, Zhou J, Wu J, Shao C. Downregulation of miR-132 by promoter methylation contributes to pancreatic cancer development. Carcinogenesis 2011; 32: 1183-1189.
13) Yu S, Lu Z, Liu C, Meng Y, Ma Y, Zhao W, Liu J, Yu J, Chen J. miRNA-96 suppresses KRAS and functions as a tumor suppressor gene in pancreatic cancer. Cancer Res 2010; 70: 6015-6025.
14) Marin-Muller C, Li D, Bharadwaj U, Li M, Chen C, Hodges SE, Fisher WE, Mo Q, Hung MC, Yao Q. A tumorigenic factor interactome connected through tumor suppressor microRNA-198 in human pancreatic cancer. Clin Cancer Res 2013; 19: 5901-5913.
15) Hamada S, Satoh K, Miura S, Hirota M, Kanno A, Masamune A, Kikuta K, Kume K, Unno J, Egawa S, Motoi F, Unno M, Shimosegawa T. miR-197 induces epithelial-mesenchymal transition in pancreatic cancer cells by targeting p120 catenin. J Cell Physiol 2013; 228: 1255-1263.
16) Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res 2002; 30: 207-210.
17) Dweep H, Sticht C, Pandey P, Gretz N. miRWalk--database: prediction of possible miRNA binding sites by "walking" the genes of three genomes. J Biomed Inform 2011; 44: 839-847.
18) Luo Z, Zhang L, Li Z, Li X, Li G, Yu H, Jiang C, Dai Y, Guo X, Xiang J, Li G. An in silico analysis of dynamic changes in microRNA expression profiles in stepwise development of nasopharyngeal carcinoma. BMC Med Genomics 2012; 5: 3.
19) Lionetti M, Biasiolo M, Agnelli L, Todoerti K, Mosca L, Fabris S, Sales G, Deliliers GL, Bicciato S, Lombardi L, Bortoluzzi S, Neri A. Identification of microRNA expression patterns and definition of a microRNA/mRNA regulatory network in distinct molecular groups of multiple myeloma. Blood 2009; 114: e20-26.
20) Enerly E, Steinfeld I, Kleivi K, Leivonen SK, Aure MR, Russnes HG, Ronneberg JA, Johnsen H, Navon R, Rodland E, Makela R, Naume B, Perala M,

Kallioniemi O, Kristensen VN, Yakhini Z, Borresen-Dale AL. miRNA-mRNA integrated analysis reveals roles for miRNAs in primary breast tumors. PLoS One 2011; 6: e16915.

21) Wang HQ, Gu B, Yi Y, Dong BY, Zhao YD, Xuan Y, Li SY, Jiang WX, Ma J. Study of regulatory pathway of related molecules in hemolytic uremic syndrome. Eur Rev Med Pharmacol Sci 2014; 18: 2886-2894.

22) He TL, Zheng KL, Li G, Song B, Zhang YJ. Identification of typical miRNAs and target genes in hepatocellular carcinoma by DNA microarray technique. Eur Rev Med Pharmacol Sci 2014; 18: 108-116.

23) Altermann E, Klaenhammer TR. PathwayVoyager: pathway mapping using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. BMC Genomics 2005; 6: 60.

24) Tabas-Madrid D, Nogales-Cadenas R, Pascual-Montano A. GeneCodis3: a non-redundant and modular enrichment analysis tool for functional genomics. Nucleic Acids Res 2012; 40: W478-483.

25) Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003; 13: 2498-2504.

26) Yang TS, Yang XH, Chen X, Wang XD, Hua J, Zhou DL, Zhou B, Song ZS. MicroRNA-106b in cancer-associated fibroblasts from gastric cancer promotes cell migration and invasion by targeting PTEN. FEBS Lett 2014; 588: 2162-2169.

27) Gong C, Qu S, Liu B, Pan S, Jiao Y, Nie Y, Su F, Liu Q, Song E. MiR-106b expression determines the proliferation paradox of TGF-beta in breast cancer cells. Oncogene 2015; 34: 84-93.

28) Dong P, Kaneuchi M, Watari H, Sudo S, Sakuragi N. MicroRNA-106b modulates epithelial-mesenchymal transition by targeting TWIST1 in invasive endometrial cancer cell lines. Mol Carcinog 2014; 53: 349-359.

29) Link A, Becker V, Goel A, Wex T, Malfertheiner P. Feasibility of fecal microRNAs as novel biomarkers for pancreatic cancer. PLoS One 2012; 7: e42933.

30) Chen WY, Liu WJ, Zhao YP, Zhou L, Zhang TP, Chen G, Shu H. Induction, modulation and potential targets of miR-210 in pancreatic cancer cells. Hepatobiliary Pancreat Dis Int 2012; 11: 319-324.

31) Huang X, Ding L, Bennewith KL, Tong RT, Welford SM, Ang KK, Story M, Le QT, Giaccia AJ. Hypoxia-inducible mir-210 regulates normoxic gene expression involved in tumor initiation. Mol Cell 2009; 35: 856-867.

32) Zhou L, Zhang WG, Wang DS, Tao KS, Song WJ, Dou KF. MicroRNA-183 is involved in cell proliferation, survival and poor prognosis in pancreatic ductal adenocarcinoma by regulating Bmi-1. Oncol Rep 2014; 32: 1734-1740.

33) Guo R, Wang Y, Shi WY, Liu B, Hou SQ, Liu L. MicroRNA miR-491-5p targeting both TP53 and Bcl-XL induces cell apoptosis in SW1990 pancreatic cancer cells through mitochondria mediated pathway. Molecules 2012; 17: 14733-14747.

34) Lai KW, Koh KX, Loh M, Tada K, Subramaniam MM, Lim XY, Vaithilingam A, Salto-Tellez M, Iacopetta B, Ito Y, Soong R, Singapore Gastric Cancer C. MicroRNA-130b regulates the tumour suppressor RUNX3 in gastric cancer. Eur J Cancer 2010; 46: 1456-1463.

35) Malzkorn B, Wolter M, Liesenberg F, Grzendowski M, Stuhler K, Meyer HE, Reifenberger G. Identification and functional characterization of microRNAs involved in the malignant progression of gliomas. Brain Pathol 2010; 20: 539-550.

36) Wu X, Weng L, Li X, Guo C, Pal SK, Jin JM, Li Y, Nelson RA, Mu B, Onami SH, Wu JJ, Ruel NH, Wilczynski SP, Gao H, Covarrubias M, Figlin RA, Weiss LM, Wu H. Identification of a 4-microRNA signature for clear cell renal cell carcinoma metastasis and prognosis. PLoS One 2012; 7: e35661.

37) Dong P, Karaayvaz M, Jia N, Kaneuchi M, Hamada J, Watari H, Sudo S, Ju J, Sakuragi N. Mutant p53 gain-of-function induces epithelial-mesenchymal transition through modulation of the miR-130b-ZEB1 axis. Oncogene 2013; 32: 3286-3295.

38) Zhao G, Zhang JG, Shi Y, Qin Q, Liu Y, Wang B, Tian K, Deng SC, Li X, Zhu S, Gong Q, Niu Y, Wang CY. MiR-130b is a prognostic marker and inhibits cell proliferation and invasion in pancreatic cancer through targeting STAT3. PLoS One 2013; 8: e73803.

39) Yamada H, Sakamoto H, Taira M, Nishimura S, Shimosato Y, Terada M, Sugimura T. Amplifications of both c-Ki-ras with a point mutation and c-myc in a primary pancreatic cancer and its metastatic tumors in lymph nodes. Jpn J Cancer Res 1986; 77: 370-375.

40) Li YJ, Wei ZM, Meng YX, Ji XR. Beta-catenin upregulates the expression of cyclinD1, c-myc and MMP-7 in human pancreatic cancer: relationships with carcinogenesis and metastasis. World J Gastroenterol 2005; 11: 2117-2123.

41) Liao DJ, Wang Y, Wu J, Adsay NV, Grignon D, Khanani F, Sarkar FH. Characterization of pancreatic lesions from MT-tgf alpha, Ela-myc and MT-tgf alpha/Ela-myc single and double transgenic mice. J Carcinog 2006; 5: 19.

42) Lewis BC, Klimstra DS, Varmus HE. The c-myc and PyMT oncogenes induce different tumor types in a somatic mouse model for pancreatic cancer. Genes Develop 2003; 17: 3127-3138.