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Integrative microRNA-mRNA and protein-protein interaction analysis in pancreatic neuroendocrine tumors

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Abstract. – OBJECTIVE: Pancreatic neuroendocrine tumors are relatively rare pancreatic neoplasms over the world. Investigations of the molecular biology of PNETs are insufficient for nowadays. We aimed to explore the expression of microRNA and messenger RNA and regulatory processes underlying pancreatic neuroendocrine tumors.

MATERIALS AND METHODS: The messenger RNA and microRNA expression profile of GSE43796 were downloaded, including 6 samples with pancreatic neuroendocrine tumors and 5 healthy samples. First, the Limma package was utilized to distinguish the differentially expressed messenger RNA and microRNA separately. Then we used microRNA Walk databases to predict the target genes of the differentially expressed microRNAs (DE-microRNAs), and selected differentially expressed target genes whose expression was reversely correlated with microRNAs. Gene Ontology classification and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis were performed to explore the functions and pathways of target genes. In addition, we constructed a regulatory miRNAs-target genes network and a protein-protein interaction network.

RESULTS: There were 28 differentially expressed microRNAs and 859 differentially expressed messenger RNAs, including 253 potential target genes. These target genes mainly enriched in ABC transporters pathway. In this network, hsa-miR-7-2-3p demonstrated the highest connectivities whereas KLF12 was the mRNAs with the highest connectivities. CXCL12 was identified as the hub of the protein-protein interaction sub-network.

CONCLUSIONS: The genes involved in ABC transporters and Type II diabetes mellitus pathway, KLF12 and CXCL12 may play an important role in the progression of pancreatic neuroendocrine tumors. However, more experimental studies are required.

Key Words:
microRNA, mRNA, Pancreatic neuroendocrine tumors, Protein-protein interaction network.

Introduction

Pancreatic neuroendocrine tumors (PNETs), also called islet cell tumors, are relatively rare pancreatic neoplasms, arising from the endocrine tissues rather than glandular epithelium of the pancreas. With secreting a variety of peptide hormones, PNETs are terminologically divided as insulinoma, gastrinoma, glucagonoma, somatostatinoma and vasoactive intestinal peptide tumors (VIPoma), resulting in numbers of clinical syndromes. However, more commonly, PNETs appear as nonfunctioning tumors[1]. The incidence of PNETs, which is less than 0.2 per 100,000 populations in 1973, has been remarkably increased during the recent decades[2]. The outcome with PNETs differs among individuals. Some of them are indolent, while others are quite aggressive. The treatment strategy, tumor-size or TNM stage and histologic grade have been reported as prognosis factors in some studies[3-5]. The only curative treatment of PNETs is radical surgery, while somatostatin analogs and chemotherapy are chosen in unresectable cases[6,7].

Though there are several studies for molecular biology of PNETs in the last decade[8-10], its unclear pathogenesis and pathophysiology need more investigations. MicroRNAs (miRNAs) are a class of non-coding RNAs with 21 and 25 nucleotides in length and function as regulators of gene expression[11]. As known, miRNAs affect in various human cancers as cancer suppressors or oncogenes[12].

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Not unexpectedly, several miRNAs alter PNETs' proliferation and/or migration[13,14]. For example, Roldo et al[14] suggested alteration in microRNA expression is related to PNETs and Kang et al[13] reported that the miR-27b would be a prognostic marker of recurrence in resected PNETs. In order to investigate the pathogenesis of solid pseudopapillary neoplasm, Park et al[15] characterized the mRNA and miRNA expression profiles of solid pseudopapillary neoplasm through comparing them with other pancreatic tumors including PNETs. However, the author did not described miRNA-mRNA crosstalk in PNETs specifically while Wang et al[16] only described the complete expression characteristics existing in PNETs. So we revealed the miRNA-mRNA crosstalk in PNETs by integrating transcriptome analysis, in order to give more detailed overviews for PNETs.

In our study, we mined a classical expression profile of miRNAs genes to construct a novel miRNA-mRNA regulatory network in PNETs. We also conducted a protein-protein interaction (PPI) network to identify the interactive associations between the target genes. Our data may contribute to future investigations for mechanisms of PNETs.

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 neuroendocrine tumors[17]{Edgar, 2002 #833}. The mRNA and miRNA expression profile of GSE43796 [the Gene Expression Omnibus (GEO) accession number], collected by Kim[15], was downloaded. The expression profiles in 6 patients with PNETs and 5 healthy control subjects were available.

Identification of DE-miRNAs and DE-mRNAs

The expression data were processed using limma package in R software, which includes background correction, quantile normalization, and final probe summarization[18]. The average expression value was used when several probes were corresponded to the same gene. We selected differentially expressed mRNA with criterion of p-value < 0.01 and |log2fold change (FC)| ≥ 2 and differentially expressed miRNA with criterion of p-value < 0.01 and |log2fold change (FC)| ≥ 2.

Identification of Differentially Expressed miRNA Target Genes

miRWalk databases (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/) were used to predict the target genes of the DE-miRNAs[19]. We predicted putative targets of differentially expressed miRNAs by six bioinformatics algorithms (DIANAmT, miRanda, miRDB, miRWalk, PICTAR and Targetscan) and recorded the miRNAs from at least 4 of 6 algorithms. We also selected miRNA target genes with experiment validation to compare with the gene list of DE-mRNAs. Because miRNAs usually down-regulate the expression of their target genes, we selected differentially expressed target genes whose expression was reversely correlated with miRNAs for subsequent investigation[20-23].

Function Annotation and Pathway Enrichment Analysis

To understand the significance of these miRNA target genes, we performed the Gene Ontology (GO) classification[24]. We also conducted the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis to search the possible pathway of miRNA target genes[25]. The Database for Annotation, Visualization and Integrated Discovery (DAVID) was utilized in these functional annotation[26].

Regulatory Network Between miRNAs and Their Targets

We construct a regulatory network among identified miRNAs-target interacting pairs. In the network, the expression of miRNA-target gene interacting pairs correlates reversely in PNETs. Cytoscape software was utilized for visualization.

PPI Network Construction and Sub-Network Mining

STRING version 10.0 comprises of > 1,100 completely sequenced organisms[27]. To identify the interactive associations between the target genes, the target genes of DE-miRNAs were input into STRING. The Cytoscape software was used to visualize these associations and the mined modules.

Results

DE-miRNAs and DE-mRNAs in PNETs

We performed differentially expressed analysis between PNETs and normal control samples. 28 miRNAs were regarded as significantly differen-

tially expressed under the threshold of p-value < 0.01 and |log2fold change (FC)| ≥ 2, with 18 up-regulated and 10 down-regulated miRNAs, as shown in Table I and Figure 1. 485 up-regulated and 374 down-regulated genes were identified to be differentially expressed in PNETs under the threshold of p-value < 0.01 and |log2fold change (FC)| ≥ 2.

Identification of Differentially Expressed miRNA Target Genes

mRNA and miRNA expression data with miRNA target predictions were combined to obtain genuine miRNA targets[20]. We input 28 miRNAs into the miRWalk database, of which 7 miRNAs are unavailable. As a result, 21 miRNAs and 253 genes formed 460 miRNA-target gene pairs with an inverse correlation of expression (Table II). 351 miRNA-target gene pairs were identified for the up-regulated miRNA, with 27 miRNA-target gene pairs validated by experiments. 109 miRNA-target gene pairs were identified for the down-regulated miRNA with 2 validated miRNA-target gene pairs.

Function Annotation and Pathway Enrichment Analysis

Generation of a signal involved in cell-cell signaling (GO:0003001, p = 3.58E-06) and neuron projection morphogenesis (GO:0048812, p = 6.09E-05) were significantly enriched for biological processes, while for molecular functions were kinase regulator activity (GO:0019207, p = 4.54E-05) and protein kinase regulator activity (GO:0019887, p = 1.33E-04), and for cellular component were synapse (GO:0045202, p = 3.95E-06) and neuron projection (GO:0043005, p = 9.62E-06) (Table III).

We used hypergeometric test with p value < 0.05 as the criteria for KEGG pathway detection. The most significant pathway in our analysis was pathways in ABC transporters (p = 4.17E-03). Furthermore, Type II diabetes mellitus (p = 3.36E-02) are also highly enriched.

Regulatory Network Between miRNAs and Their Targets

Using the 460 miRNA-target gene pairs, a miRNA-target gene regulatory network was con-

Table I. Differentially expressed microRNAs.

	MicroRNA	logFC	*p*-value
Up-regulated microRNAs	hsa-miR-7-5p	5.890721054	5.17165E-08
	hsa-miR-129-1-3p	4.9827617	1.15467E-06
	hsa-miR-95	3.437924319	1.98996E-06
	hsa-miR-301a-3p	3.224740755	2.23728E-06
	hsa-miR-129-2-3p	6.112467723	3.46129E-06
	hsa-miR-153	3.326933352	7.15502E-06
	hsa-miR-129-5p	4.756540337	1.37489E-05
	hsa-miR-7-2-3p	2.067017435	1.60769E-05
	hsa-miR-148b-3p	2.220145552	1.64837E-05
	hsa-miR-98	2.313555307	2.2927E-05
	hsa-miR-324-5p	2.16861939	4.33488E-05
	hsa-miR-744-5p	2.06162945	5.46663E-05
	hsa-miR-652-3p	2.365240259	7.88717E-05
	hsa-miR-99b-5p	2.157139096	0.00012127
	hsa-miR-183-5p	2.958884704	0.000990997
	hsa-miR-182-5p	2.20010559	0.001506595
	hsa-miR-429	2.144472745	0.001994266
	hsa-miR-598	2.113267622	0.003581726
Down-regulated microRNAs	hsa-miR-21-3p	-2.28275003	0.001123236
	hsa-miR-216b	-4.597941027	0.001760796
	hsa-miR-217	-4.503620232	0.002328638
	hsa-miR-146b-5p	-2.683441954	0.002879437
	hsa-miR-216a	-4.667124297	0.002888823
	hsa-miR-199b-5p	-2.660497696	0.004966733
	hsa-miR-144-3p	-2.302352291	0.005137975
	hsa-miR-4299	-2.180646903	0.005224459
	hsa-miR-1225-5p	-2.263195271	0.006813705
	hsa-miR-148a-3p	-2.826426344	0.009230868

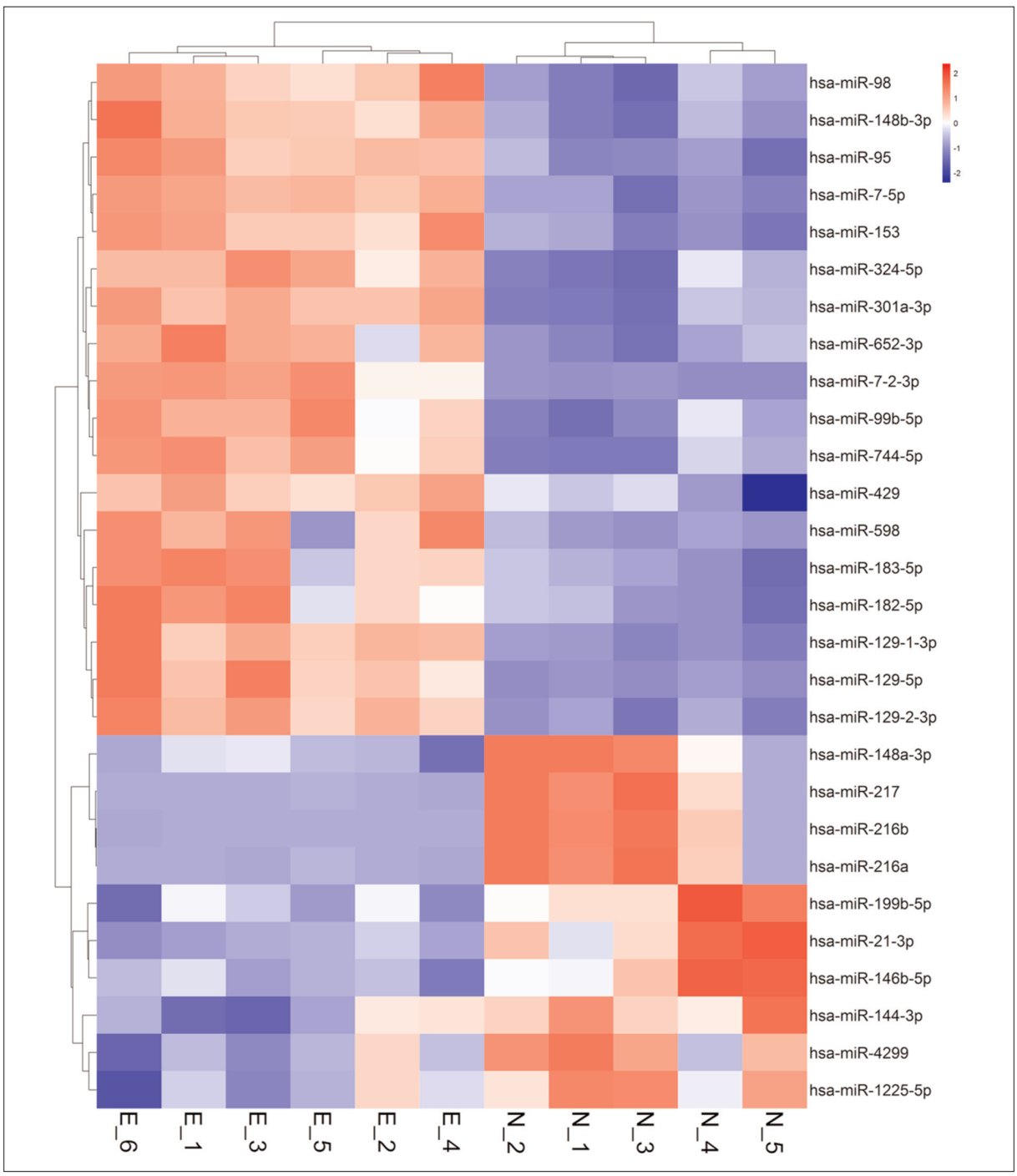

Figure 1. Heat map of differentially expressed miRNAs. E_(1-6): patient with PNETs; N_(1-5): healthy control people. The expression of differentially expressed microRNA between PNETs and healthy controls are shown in different colors, from blue to red, meaning increasing expression.

structed (Figure 2). In this network, hsa-miR-7-2-3p, hsa-miR-429, hsa-miR-182-5p, hsa-miR-129-5p and hsa-miR-148b-3p demonstrated the highest connectivity, which regulate 72, 44, 37, 35, and 35 targets respectively. KLF12, NFASC, PKIA, RAB3B and IDS were the mRNAs with the highest connectivity, which were affected by 7, 6, 6, 6 and 5 miRNAs.

Table II. Target genes of differentially expression microRNAs.

MicroRNA	Counts	Target mRNAs
Up-regulated microRNAs		
hsa-miR-7-5p	21	COX11, ENO2, FBXL16, FMN2, GPR19, ICA1L, IDS, KCNH2, KLF12, PDE4D, RFX2, RNF41, SGIP1, SNCA, STMN3, TFF3, TMX4, TOX, TSPYL4, WFS1, ZNF365,
hsa-miR-129-1-3p	21	B9D1, BSN, CACNA1C, CDK5R1, CDS1, KLF12, MAPK10, NFASC, NMNAT2, OSBPL10, PKIA, PPP1R14C, PRKCE, RCAN2, RNF34, SLC6A17, SYT4, TMCC3, TPD52, UQCC1, ZNF365
hsa-miR-301a-3p	26	ARX, CDK5R1, CDS1, HABP4, HECW2, KIF5C, KLF12, LRCH2, MAPK10, MYT1, NAP1L3, NOL4, OPA3, RAB9B, RAPGEF4, RIMBP2, RTN1, SLC24A3, SLC7A14, SMOC1, SNAP25, SPIRE1, ST18, ST8SIA3, TMOD2, ZC3H12B,
hsa-miR-129-2-3p	21	B9D1, BSN, CACNA1C, CDK5R1, CDS1, KLF12, MAPK10, NFASC, NMNAT2, OSBPL10, PKIA, PPP1R14C, PRKCE, RCAN2, RNF34, SLC6A17, SYT4, TMCC3, TPD52, UQCC1, ZNF365
hsa-miR-129-5p	35	ABCA5, ABCB1, ABCC5, ANGPT2, CACNA1A, CACNA1C, CAMK2N1, CSRNP3, FAM46A, HDGFRP3, KATNAL1, KIAA1377, KIF5C, KLF12, LPPR4, MTMR7, MYT1, NFASC, NOL4, OPA3, PCLO, PCSK2, PKIB, PRKCE, PTPRN, RAB3B, RIMS2, SAMD5, SH3GL3, SLC7A14, SNCA,
hsa-miR-7-2-3p	72	SPIRE1, ST8SIA3, TOX, TSPYL4 ABCA5, ABCG1, AP3M2, CACNA1C, CAMK2B, CDH10, CPNE4, CSRNP3, ESM1, FAM131A, FAM167A, FAM46A, FGF13, FGF14, FHDC1, HABP4, HECW2, IDS, INA, JAKMIP2, JAZF1, KATNAL1, KCNJ6, KIF5C, KLF12, LIMCH1, LPPR4, LRCH2, MAPK10, MTMR7, MUC1, MVB12B, NAAA, NAP1L2, NETO2, NFASC, NMNAT2, NOVA1, OSBPL10, PDE4D, PFDN4, PIPOX, PKIA, PLAC8, PPP4R4, PRKAR1A, PRUNE2, QDPR, RAB39B, RAB3B, RAB6B, RGAG4, RIMBP2, RUFY3, RUNDC3B, SCG3, SCHIP1, SLC12A5, SLC7A14, SNAP91, SNCA, SPTSSB, ST18, SYT13, SYT4, TMOD2, TOX, TPD52, TRPC1, TUSC3, ZNF226, ZSCAN2
hsa-miR-148b-3p	35	ANGPT2, B4GALT6, BPGM, C1GALT1, CDK5R1, CDS1, CEACAM1, CPNE4, H2AFY, HECW2, HMGB3, JPH3, KCNJ6, MAPK10, MUC13, MYO9A, NAT14, NME5, NOL4, PBXIP1, PCSK1, PDE4D, PPP2R2C, RAB3B, RNF150, SLC24A3, SLC7A14, SNAP91, SNCA, SPIRE1, ST18, ST8SIA3, SYT4, TMCC3, ZC3H12B
hsa-miR-324-5p	8	ARNT2, CDS1, CKB, LHFPL4, NAPB, PRKCE, RAB3B, RGAG4
hsa-miR-744-5p	8	CALY, CKB, EEF1A2, HIST1H2BD, LPHN1, SPTBN4, SYT7, TSPAN7
hsa-miR-652-3p	1	HIST1H2BD
hsa-miR-99b-5p	1	ENO2
hsa-miR-183-5p	21	ACTR1A, AP3M2, CDK5R1, CELSR3, CSRNP3, DYNC1I1, EFR3B, FHDC1, IDS, KIF5CNMNAT2, PDE4D, PKIA, PRKAR1A, RAB9B, RAPGEF4, RIMBP2, RNF41, SLC6A17, ST8SIA3, SV2A,
hsa-miR-182-5p	37	CACNA2D2, CAMK2N1, CDHR3, CNTN1, CSRNP3, CYCS, FAM133A, FAM167A, IDS, JAKMIP2, JAZF1, KIF5C, LPHN1, LPPR4, MVB12B, NCALD, NFASC, OSBPL10, PDZD4, PEG10, PKIA, PRKAR1A, PRUNE2, RAB3B, RAB6B, RAB9B, RAPGEF4, RGS17, RIMBP2, RUNDC3B, SAMD5, SLC7A14, SPIRE1, TMCC3, TMEM145, TOX, TPD52
hsa-miR-429	44	ABCA5, AFF3, B4GALT6, CACNA1C, CNTN1, COX11, CSRNP3, DYNC1I1, ESM1, FBXL16, GABARAPL2, HMGB3, ICA1L, IDS, ITGA1, JAKMIP2, JAZF1, KATNAL1, KIAA1244, KLF12, LIN7B, LPPR1, LPPR4, MYO9A, MYT1, NAP1L2, NAP1L5, NAPB, NFASC, NOVA1, PCSK2, PFDN4, PKIA, PPP2R2C, PRKAR1A, RAB3B, RAB9B, RIMS2, SCHIP1, SLC12A5, SLC6A17, SLC8A3, SNAP25, STX1A
Down-regulated microRNAs		
hsa-miR-21-3p	14	BACE1, CLDN1, ENPP1, GAS1, GCOM1, KIAA1522, NFIB, PHF2, PRR14L, SDC4, SLC4A4, SNTB1, TMEM164, ZNF704
hsa-miR-217	14	BICC1, CACHD1, CFTR, CXCL2, GRHL2, HNF1B, MED13, PARVA, PBX1, SEL1L, SLC4A4, STEAP3, WDR72, ZNF704
hsa-miR-146b-5p	13	AFAP1L2, AQP1, BTG2, C1orf106, C9orf72, CNTFR, HEYL, LFNG, MED13, SLC2A10, TGIF1, TRIM5, ZNF704
hsa-miR-199b-5p	12	BICC1, BTG2, CMKLR1, ENPP1, KIAA1522, PDCD4, RGMA, SERPINA5, STEAP3, TMEM164, TMEM97, TSPAN6
hsa-miR-144-3p	30	BICC1, C9orf72, CACHD1, CAMTA2, CAV2, CEBPA, CFTR, CXCL11, CXCL12, DMD, EDN1, FAM129A, FOSB, GDF10, HNF, IFFO2, LGR4, MED13, MGST1, MXRA5, PARVA, PRDM16, RGMA, SEL1L, SHANK2, SLC25A15, SLC39A14, TGIF1, ZFP36L2, ZNF704
hsa-miR-1225-5p	8 18	ACSS1, BACE1, CXCL17, IQGAP2, MED13, PLTP, SEL1L3, SOX9 ADM2, AKAP7, BICC1, CCKBR, CXCL12, DMRTA2, FOSB, GAS1, MTMR3, PKNOX2, PPAP2C, PRDM16, PXMP2, RDH10, RGMA, SHANK2, TMEM97, ZNF704

Table III. GO functional annotation for differentially expression miRNA target genes. (Top 10).

GO ID	GO Term	Count	*p*-value
Biological process			
GO:0003001	Generation of a signal involved in cell-cell signaling	10	3.58E-06
GO:0048812	Neuron projection morphogenesis	13	6.09E-05
GO:0031175	Neuron projection development	14	8.76E-05
GO:0007269	Neurotransmitter secretion	6	1.17E-04
GO:0019226	Transmission of nerve impulse	16	1.68E-04
GO:0006836	Neurotransmitter transport	8	1.91E-04
GO:0048858	Cell projection morphogenesis	13	2.28E-04
GO:0030072	Peptide hormone secretion	6	2.29E-04
GO:0015674	Di-, tri-valent inorganic cation transport	11	2.33E-04
GO:0016192	Vesicle-mediated transport	21	2.48E-04
Cellular component			
GO:0045202	Synapse	19	3.95E-06
GO:0043005	Neuron projection	18	9.62E-06
GO:0043025	Cell soma	11	1.65E-04
GO:0005626	Insoluble fraction	27	1.86E-04
GO:0005624	Membrane fraction	26	2.61E-04
GO:0044459	Plasma membrane part	51	5.31E-04
GO:0000267	Cell fraction	30	8.78E-04
GO:0030054	Cell junction	18	0.001395848
GO:0019717	Synaptosome	7	0.001438754
GO:0042995	Cell projection	21	0.002671632
Molecular function			
GO:0019207	Kinase regulator activity	9	4.54E-05
GO:0019887	Protein kinase regulator activity	8	1.33E-04
GO:0000149	SNARE binding	6	1.51E-04
GO:0008092	Cytoskeletal protein binding	19	2.39E-04
GO:0003779	Actin binding	13	0.002068596
GO:0005509	Calcium ion binding	24	0.004474706
GO:0030276	Clathrin binding	3	0.005121461
GO:0019905	Syntaxin binding	4	0.008992828
GO:0005516	Calmodulin binding	7	0.013643713
GO:0030295	Protein kinase activator activity	3	0.025544056

[a]Count = the number of differentially expression miRNA target genes involved in GO terms.

Interaction Network Construction and Module Analysis

We input the target genes of 21 DE-miRNAs into the STRING database. The significant interactions with a confidence score of > 0.4 were utilized to generate a protein-protein interaction (PPI) network by using Cytoscape software (Figure 3 A). Five nodes with high degree were MGST1, CEACAM1, PDZD4, AP3M2 and MTMR7. Among them, MGST1 were upregulated while the other 4 genes were down-regulated. Because the PPI network contains numerous nodes, it is difficult to select the useful information. Therefore, the modules were mined in the PPI network. As a result, a PPI sub-network with 12 target nodes was constructed, including CXCL12, PRKAR1A, CFTR, EDN1, SDC4, FGF13, CXCL11, CXCL2, CCKBR, ITGA1, DMD and SNTB1. As shown in Figure 3 B, CXCL12 was the hub protein. The KEGG pathway analysis demonstrated these genes associated with chemokine signaling pathway and cytokine-cytokine receptor interaction.

Discussion

In the current study, we identified 28 differentially expressed miRNAs and 859 differentially expressed mRNAs. 253 miRNA target genes whose expression correlates reversely with that of corresponding miRNAs in PNETs. We also constructed a regulatory network with 460 miRNA-target gene pairs. Through PPI network construction and module mining, a CXCL12 module was formed and were identified significantly in PNETs.

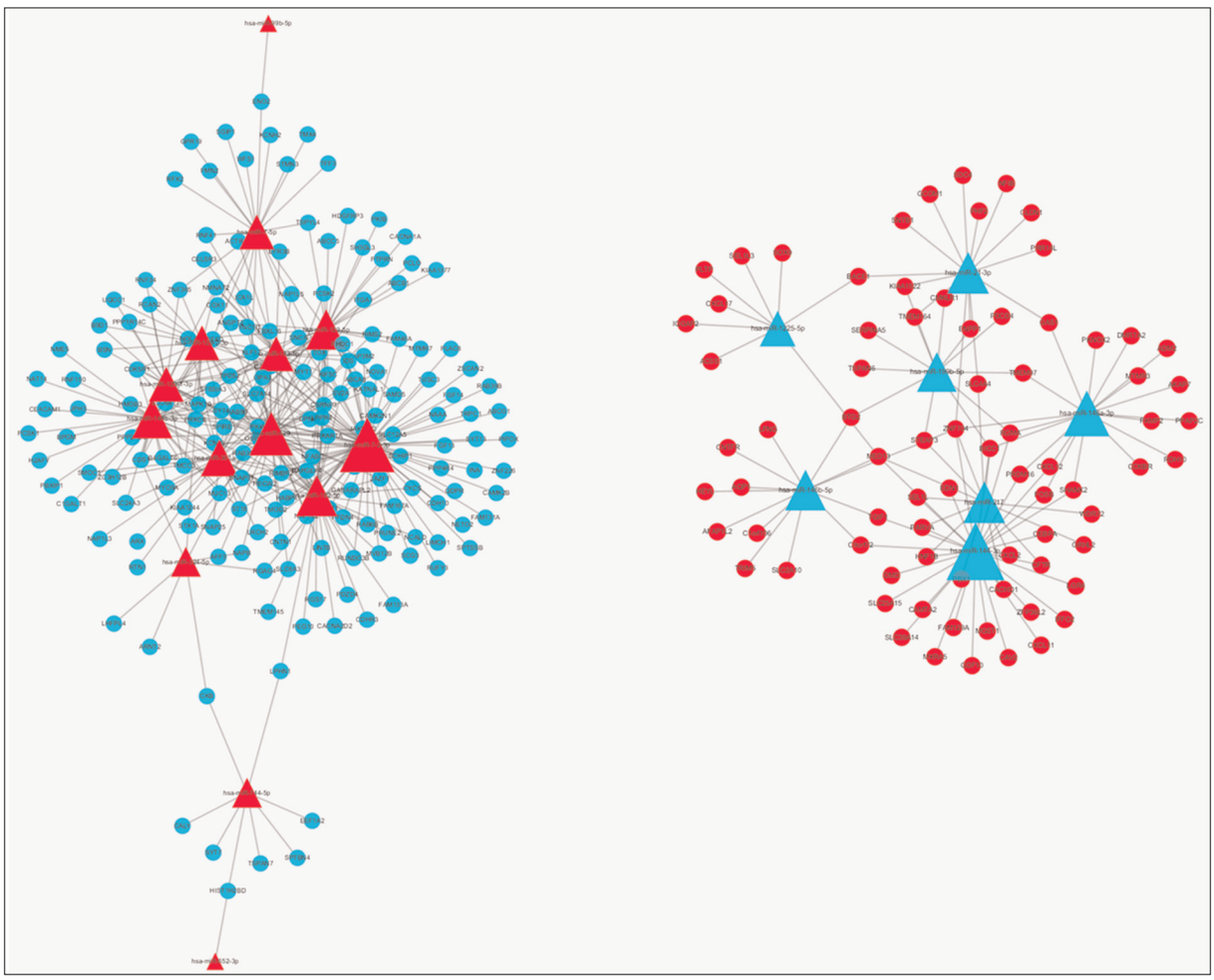

Figure 2. microRNA-target genes interaction network in pancreatic neuroendocrine tumors. microRNAs and target genes are replaced by triangles and ellipses respectively. The blue indicates the low expression while the red represents high expression. The larger drawing size represents more microRNAs or genes interactions with it.

In the miRNA profile, most of DE-miRNAs in our work were identified in the progression of PNETs and other cancers. hsa-miR-183 dysregulates cell proliferation by down-regulating the Bmi-1 expression in pancreatic cancer[28]. has-miR-429 determines poor outcome and inhibits pancreatic ductal adenocarcinoma growth by targeting TBK1[29]. The frequently down-regulated hsa-miR-217 can regulate KRAS and function as a tumor suppressor in PDAC[30]. The hsa-miR-144 family promotes cell proliferation via targeting PTEN/AKT pathway in insulinomas[31]. The hsa-miR-182-5p inhibited IGF-1R mRNA expression in hepatocellular carcinoma[32]. The hsa-miR-129-5p may directly inhibit STAT3 expression and affect the development of laryngeal squamous cell carcinoma[33]. The overexpression of hsa-miR-7-5p inhibited cell proliferation and induced apoptosis by mainly targeting REG in breast cancer cells.

KEGG pathway enrichment analysis showed that target genes significantly enriched in ABC transporters and Type II diabetes mellitus pathways, containing many genes including ABCB1, CFTR, ABCG1, MAPK10, CACNA1C, PRKCE and CACNA1A. These related genes were mainly targeted by hsa-miR-129-5p, hsa-miR-129-2-3p, hsa-miR-217, hsa-miR-324-5p and hsa-miR-7-2-3p. ABC transporters utilize the energy of ATP binding and hydrolysis to transport various substrates across cellular membranes, which is related to the mechanisms of drug resistance in cancer. Mohelnikova-Duchonova et al[34] identified that the expression of ABC transporters was significantly

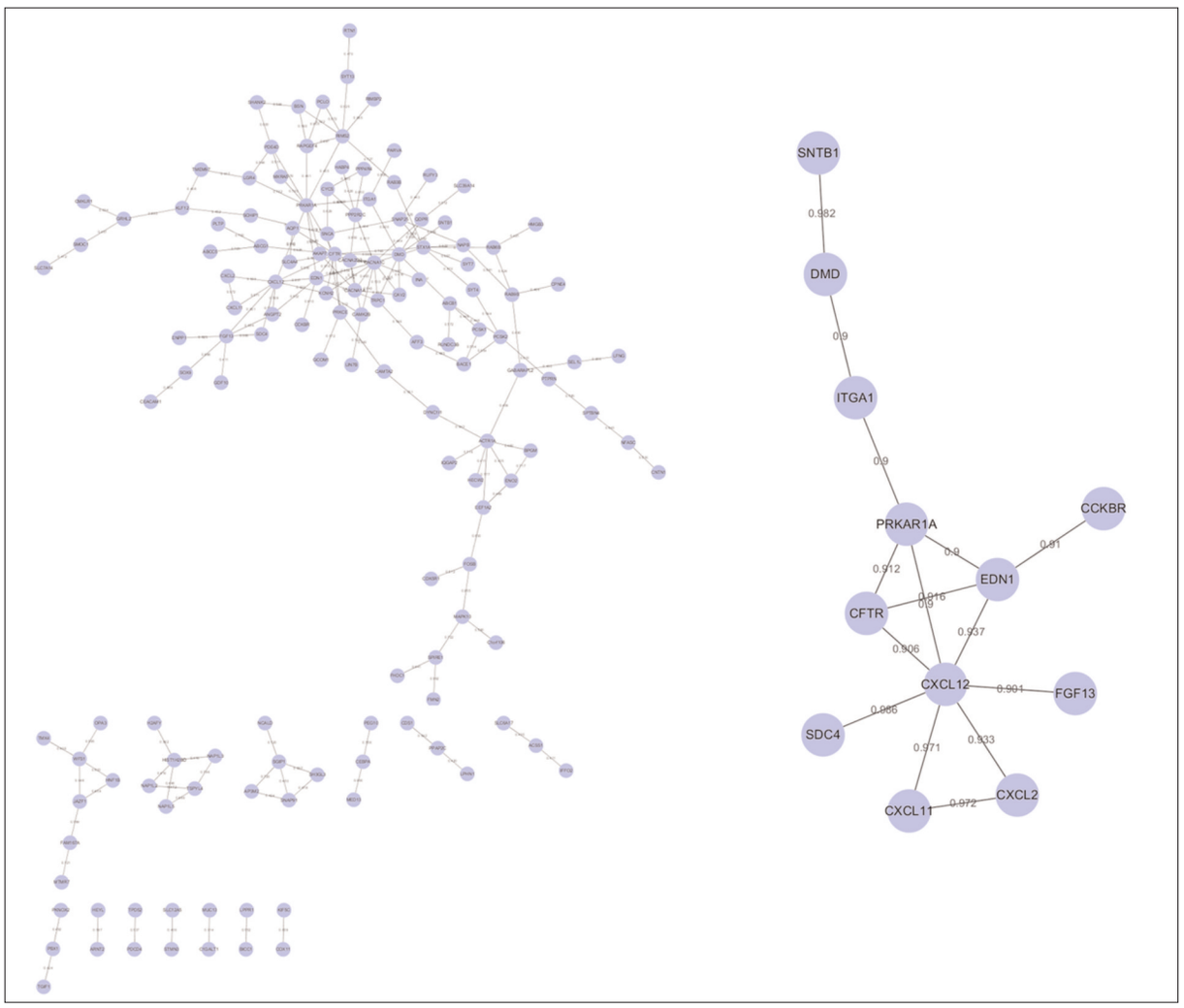

Figure 3. Protein-protein interaction (PPI) network of target genes ***(A)*** and the sub-network in PPI network ***(B)***. The confidence scores are marked in the line of interaction. CXCL12 was the hub protein in the sub-network.

dysregulated in pancreatic tumor when compared to normal tissue. Santisteban[35] suggested that ABC transporters functioned as molecular effectors of pancreatic oncogenic pathways by the hedgehog-GLI model. Type 2 diabetes is closely associated with pancreatic cancer[36], which was also shown in a clinical epidemiological studies[37]. In a case reported by Yu et al[37], a patient with an inactive mutation of glucagon receptor showed hyperglucagonemia and hyperplasia of pancreatic α cells, and finally developed PNETs. In a word, the target genes involved in ABC transporters and Type 2 diabetes pathways may play an important role triggering the tumorigenesis of PNETs.

In the miRNA-target gene regulatory network, hsa-miR-7-2-3p demonstrated the highest connectivities whereas KLF12 was the mRNAs with the highest connectivities. It suggested that hsa-miR-7-2-3p and KLF12 may affect the tumorigenesis of pancreatic cancer. In the present study, we found that hsa-miR-7-2-3p up-regulates PNETs. Kefas et al[38] established hsa-miR-7 family inhibits the epidermal growth factor receptor (EGFR) and the Akt Pathway and acts as a regulator of major cancer pathways. Godin-Heymann et al[39] reported that expression of KLF12 can promote the cell cycle transition and regulate ankoikis through S phase and therefore cell proliferation. But KLF12 is down-regulated in lung cancer cell lines. Reduced expression levels of KLF12 results in increased ability of lung cancer cells to form tumors *in vivo*.

In addition, CXCL12 is the hub of the PPI sub-network, which indicated that CXCL12 has affect

the progression of PNETs. CXCL12, also known as stromal cell derived factor-1 (SDF-1), is a small molecules cytokine and its receptor is CXCR4[40]. CXCL12 is not only strongly chemotactic for lymphocytes, but also plays an important role in angiogenesis by recruiting endothelial progenitor cells (EPCs) from the bone marrow, which makes it functional in carcinogenesis and tumor neovascularisation[41-43]. CXCL12 also influences tumor metastasis by the CXCR4-CXCL12 interaction[44]. Preliminary evidence showed that the axis CXCR4-CXCL12-CXCR7 is overexpressed and functional in PNETs. Missiaglia et al[45] found that their research strongly support function for PI3K/Akt/mTOR pathway in PNETs. Moreover, the PI3K/Akt/mTOR pathway has crosstalk with the CXCR4-CXCL12-CXCR7 chemokine receptor axis, which has a crucial role in cancer progression[46]. CXCL12 is overexpressed when comparing with the normal sample in our study, and FGF13 (fibroblast growth factor 13), the new prognostic marker in PNETs mentioned in Missiaglia et al study, is downregulated in the sub PPI network. Thus CXCR4-CXCL12-CXCR7 and PI3K/Akt/mTOR pathways are expressed and functional in NETs and may represent a prognostic factor. However, more original studies are needed to confirm the results.

The present study has many limitations. First, the data we analyzed consist of only 6 PNETs and 5 normal samples, and each sample only told us the age and gender, without any clinical and pathological characteristics. So we did not further study PNETs, such as analyzing the different expression profile under different PNETs outcomes and so on. Then, dealing with the gene expression profiles, we only identified DE-mRNAs for our further research, without any thorough analysis for mRNA, which had been reported by Wang[16]. However, when prepared the paper, we could not get the same results as Wang's through limma package. This disparity may result from different analysis methods. Finally, the *p*-values of KEGG enrichment pathway enrichment analysis were relatively large, which indicated the relativity was weak.

Conclusions

28 differentially expressed miRNAs and 859 differentially expressed mRNAs were identified, including 253 potential target genes. In miRNA-target gene regulatory network, hsa-miR-7-2-3p and KLF12 demonstrated the highest connectivity and CXCL12 were identified as the hub of proteins in the PPI sub-network. The genes involved in ABC transporters and type-diabetes mellitus pathway, KLF12 and CXCL12 may affect the progression of PNETs. However, more experimental studies are required to demonstrate the role of these miRNAs in PNETs.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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