Possible Molecular Markers for the Diagnosis of Pancreatic Ductal Adenocarcinoma

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

AB Quan Shen
BC Miao Yu
B   Jiang-Kun Jia
CD Wen-Xi Li
D   Yu-Wei Tian
AE Huan-Zhou Xue

Department of Hepatobiliary Pancreatic Surgery, Henan Provincial People's Hospital, Zhengzhou, Henan, P.R. China

Corresponding Author: Huan-Zhou Xue, e-mail: hzxue17@163.com
Source of support: Departmental sources

Background: We aimed to identify pivotal genes and pathways involved in pancreatic ductal adenocarcinoma (PDAC), and explore possible molecular markers for the early diagnosis of the disease.

Material/Methods: The array data of GSE74629, including 34 PDAC samples and 16 healthy samples, was downloaded from GEO (Gene Expression Omnibus) database. Then, the DEGs (differentially expressed genes) in PDAC samples were compared with healthy samples using limma (linear models for microarray). Gene functional interaction networks were analyzed with Cytoscape and ReactomeFIViz. PPI networks were constructed with Cytoscape software. In addition, PPI (protein-protein interaction) network clustering modules were analyzed with ClusterONE, and the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analyses for modules were performed.

Results: A total of 630 upregulated and 1,002 downregulated DEGs were identified in PDAC samples compared with healthy samples. Some ribosomal protein genes with higher average correlation in module 0 were enriched in the ribosome pathway. NUP107 (nucleoporin 107 kDa) and NUP160 (nucleoporin 160 kDa) were enriched in module 3. HNRNPU (heterogeneous nuclear ribonucleoprotein U) with higher average correlation in module 8 was enriched in the spliceosome pathway. The ribosome pathway and the spliceosome pathway were significantly enriched in cluster 1 and cluster 2, respectively.

Conclusions: Ribosomal protein genes Nup170, Nup160, and HNRNPU, and the ribosome pathway as well as the spliceosome pathway may play important roles in PDAC progression. In addition, ribosomal protein genes Nup170, Nup160, and HNRNPU may be used as possible molecular markers for the early diagnosis of the disease.

MeSH Keywords: Carcinoma, Pancreatic Ductal • Chemistry, Bioinorganic • Community Networks

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/906313

🖺 2075   ⊞ 2   📊 5   📖 43

Background

Pancreatic ductal adenocarcinoma (PDAC), the most common pancreatic neoplasm, is expected to turn into the second most common cause of deaths associated with cancer by 2030 [1,2]. It is estimated that a total of 45,220 patients will be diagnosed with pancreatic cancer and 38,460 will die of this disease in the United States in 2013 [3]. Risk factors for PDAC include age, gender, ethnicity, cigarette smoking [4,5]. In addition, a very poor overall prognosis is one of the most important characteristic of PDAC, and the median survival time after diagnosis is only 3–5 months [6]. Thus, accurate early diagnosis for PDAC is needed and discovery of the molecular mechanisms may provide a new option for the early diagnosis of PDAC.

Recently, a wealth of previous studies has been applied to gain a better understanding of the molecular mechanisms of PDAC. One study showed that *SEL1L* (*Sel-1-like*) was downregulated by upregulated has-miR-155 in PDAC patients, which suggested that specific miRNAs played important roles in the pathogenesis of this disease [7]. Steele et al. suggested that microRNA (miRNA) such as miR-21 might serve as a screening tool for PDAC in the future [8]. The miR-10b is upregulated in PDAC and can be used as a diagnostic marker in suspicious pancreatic lesions [9]. Furthermore, the serum *MMP7* (matrix metalloproteinase 7) level in PDAC patients correlates with metastatic disease and survival [10]. Oji et al. indicated that *WT1* (Wilms Tumor 1) played a significant part in the tumorigenesis of PDAC and provided a new option for the treatment of this disease [11]. In addition, mutation of *TP53* (tumor protein 53) contributes to rapid progression of premalignant cells to pancreatic tumor and *FoxM1* (forkhead box M1) can be used as prognostic molecular marker for PDAC [12,13]. Besides, activation of the Jak/Stat3 (Janus kinase/signal transducer and activator of transcription 3) pathway is related with adverse outcome of PDAC after resection [14]. The Wnt pathway regulates the metastasis-promoting mucin 4 in PDAC, and then contributes to disease metastases and progression [15]. However, since these genes and pathways are not sufficient to clarify the molecular mechanisms of the PDAC, there is a need to identify more pivotal genes and pathways important in the progression of this disease.

Gene expression technologies are increasingly being used to identify DEGs (differentially expressed genes) in neoplastic tissues compared with their normal tissues [16]. In the present study, we downloaded the array data of GSE74629 and analyzed the DEGs associated with PDAC. Then, functional network analyses were performed for these DEGs. In addition, protein-protein interaction (PPI) network and important clustering modules were analyzed. We aimed to find pivotal genes and pathways involved in PDAC, and explore possible molecular markers for the early diagnosis of the disease.

Material and Methods

Microarray data

The array data of GSE74629 was downloaded from GEO (Gene Expression Omnibus, *http://www.ncbi.nlm.nih.gov/geo/*) database with the platform of GPL10558 (Illumina HumanHT-12 V4.0 expression beadchip, Jaen, Spain). A total of 34 PDAC samples and 16 healthy samples were included in this study.

Data preprocessing

The downloaded non-normalized gene expression data were preprocessed by limma (linear models for microarray) [17] in R package, including background correcting, normalizing and calculating expression. Then, the probe ID was transformed to gene symbol with illuminaHumanv4.db [18] in R annotation package and the probe that was not matched with gene symbol was eliminated. For one gene symbol mapped by several probes, the mean value of these probes was set as the final gene expression level. Finally, totally 21,318 gene expression values were obtained.

Screening of DEGs

The DEGs in PDAC samples compared with healthy samples were analyzed with limma. The *p*-values were calculated by *t*-test [19] in limma package. BH (Benjamini-Hochberg) [20] was used to adjust *p*-values into FDR (false discovery rate) values. FDR <0.05 and |log2FC (fold change)| ≥0.58 were set as cut-off criterion for DEGs.

Functional network analysis

The ReactomeFIViz app [21] can calculate correlations (as weights for edges in the whole functional interaction network) among genes involved in the same functional interactions, apply Monte Carlo localization graph clustering algorithm to the weighted functional interaction network, and form a sub-network for selected network modules on the basis of module size and average correlation.

In the present study, Cytoscape [22] and ReactomeFIViz app were used to analyze gene functional interaction network. Modules size ≥6 and average correlation ≥0.25 were set as criteria. Subsequently, pathway enrichment analyses were performed for each function module, and biological pathways involved by each module gene were observed with FDR <0.05.

PPI network analysis

STRING (Search Tool for the Retrieval of Interacting Genes) [23] is a database used to provide information of interaction of
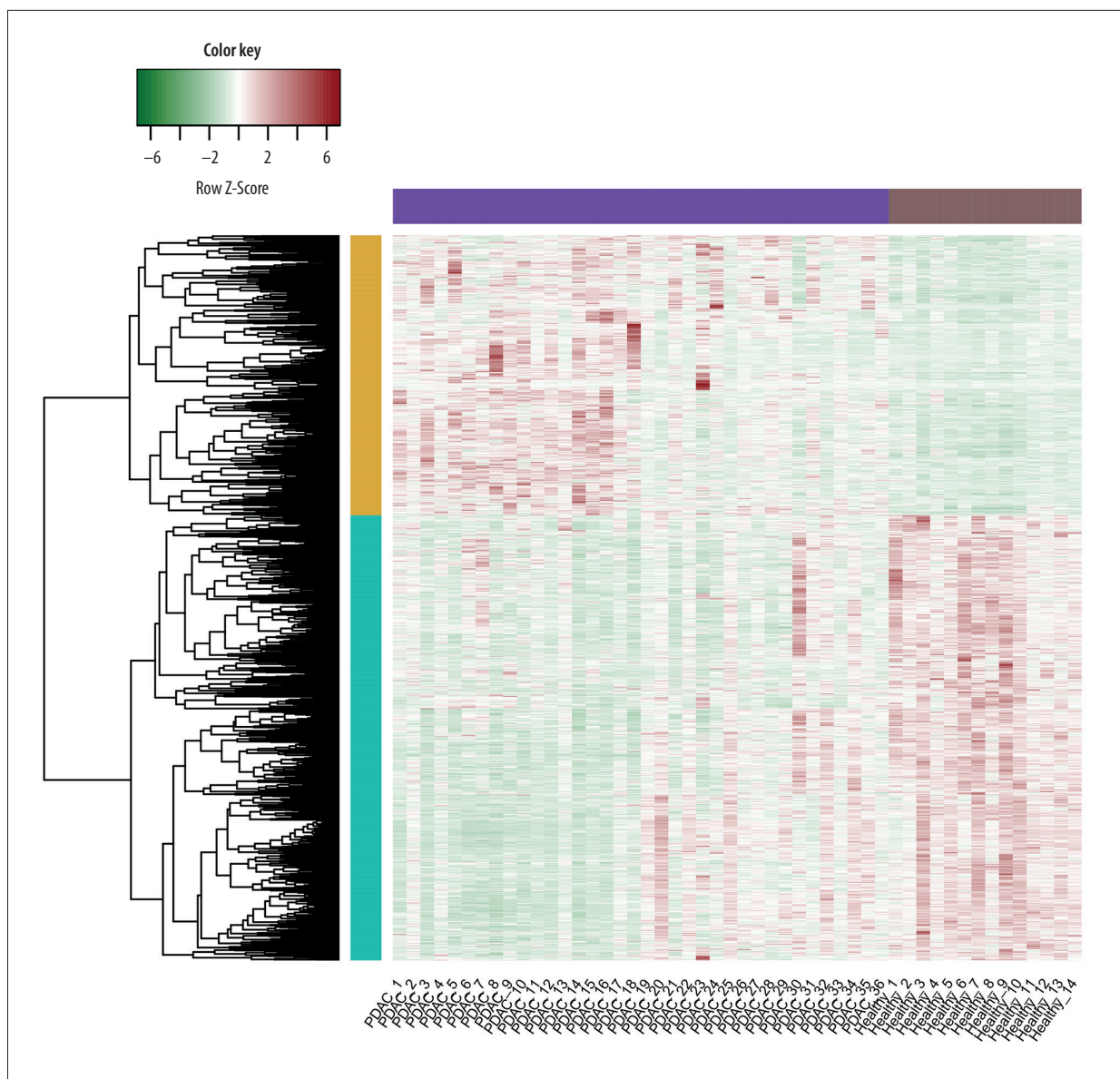
Figure 1. Heat map of differentially expressed genes (DEGs). The green represents higher expression levels; the red represents lower expression levels.

proteins, and neighborhood, gene fusion, co-occurrence, co-expression experiments, databases and text mining were prediction method of this database. In this study, the input gene sets were gene modules and species were Homo sapiens. PPI score >0.9 was set as the cutoff value and at least one protein interaction nodes was a module gene. PPI networks were constructed with Cytoscape software.

PPI network clustering modules analyses

KEGG (Kyoto Encyclopedia of Genes and Genomes) can be used to reveal functions of genes or other molecules [24]. The subnetwork modules in the PPI network tended to take part

in common biological processes. ClusterONE [25] was used to analyze network modules, and the KEGG pathway enrichment analyses for modules were performed. Modules that *p*-value <3E-4 were set as significant modules.

Results

Data processing and DEGs analysis

As shown in Figure 1, a total of 630 upregulated and 1,002 downregulated genes were differently expressed in PDAC samples compared with healthy samples.
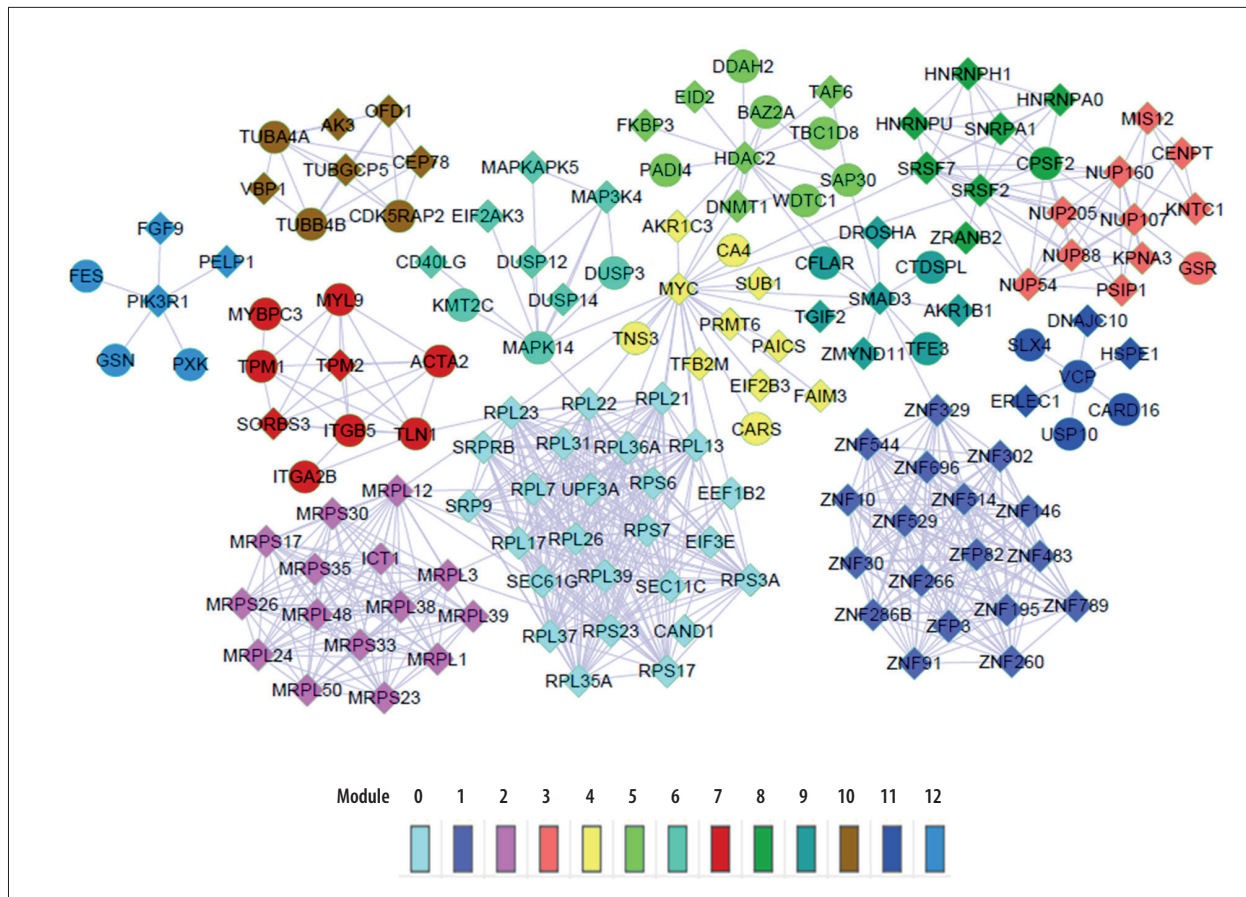
Figure 2. Functional network of differentially expressed genes (DEGs). Different colors represent different modules; diamond-shaped nodes represent downregulated genes; circular nodes represent upregulated genes.

Functional network analysis

A total of 13 subnetwork modules including 146 nodes and 684 gene pairs were included in the network (Figure 2). Particularly, the average correlation of module 0, module 3, and module 8 was greater than 0.6, which indicated strong correlation (Table 1).

Ribosomal protein genes, such as *RPL13* (ribosomal protein L13), *RPL17* (ribosomal protein L17), *RPL21* (ribosomal protein L21), *RPL22* (ribosomal protein L22), *RPL23* (ribosomal protein L23), *RPL26* (ribosomal protein L26), *RPL31* (ribosomal protein L31), *RPL35A* (ribosomal protein L35A), *RPL36A* (ribosomal protein L36A), *RPL37* (ribosomal protein L37), *RPL39* (ribosomal protein L39), *RPL7* (ribosomal protein L7), *RPS17* (ribosomal protein S17), *RPS23* (ribosomal protein S23), *RPS3A* (ribosomal protein S3A), *RPS6* (ribosomal protein S6) and *RPS7* (ribosomal protein S7) were enriched in module 0. *NUP107* (nucleoporin 107 kDa) and *NUP160* (nucleoporin 160 kDa) were enriched in module 3. *HNRNPU* (heterogeneous nuclear ribonucleoprotein U) was enriched in module 8.

Pathway analysis of functional network module and PPI network analysis

Pathways significantly enriched by each module are shown in Table 2. Ribosomal protein genes were mainly enriched in three pathways including SRP-dependent co-translational protein targeting to membrane, eukaryotic translation termination, and ribosome. *NUP107* and *NUP160* were mainly enriched in two pathways including hexose transport and metabolism of non-coding RNA. *HNRNPU* was mainly enriched in the spliceosome pathway.

The PPI network for other DEGs interacted with 13 module genes was shown in Figure 3. A total of 243 nodes and 676 interaction pairs were included in this network.

PPI network clustering modules analyses

We obtained two subnetwork modules with ClusterOne (Figure 4), and the GO (Gene Ontology) and KEGG pathways significantly enriched in two subnetwork modules are shown in Figure 5.

Table 1. Information list of 13 sub network modules.

Module	Nodes in module	Node percentage	Average correlation	Node list
0	25	0.1701	0.6255	CAND1, EEF1B2, EIF3E, RPL13, RPL17, RPL21, RPL22, RPL23, RPL26, RPL31, RPL35A, RPL36A, RPL37, RPL39, RPL7, RPS17, RPS23, RPS3A, RPS6, RPS7, SEC11C, SEC61G, SRP9, SRPRB, UPF3A
1	18	0.1224	0.4769	ZFP3, ZFP82, ZNF10, ZNF146, ZNF195, ZNF260, ZNF266, ZNF286B, ZNF30, ZNF302, ZNF329, ZNF483, ZNF514, ZNF529, ZNF544, ZNF696, ZNF789, ZNF91
2	15	0.102	0.4808	ICT1, MRPL1, MRPL12, MRPL24, MRPL3, MRPL38, MRPL39, MRPL48, MRPL50, MRPS17, MRPS23, MRPS26, MRPS30, MRPS33, MRPS35
3	11	0.0748	0.602	CENPT, GSR, KNTC1, KPNA3, MIS12, NUP107, NUP160, NUP205, NUP54, NUP88, PSIP1
4	11	0.0748	0.4621	AKR1C3, CA4, CARS, EIF2B3, FAIM3, MYC, PAICS, PRMT6, SUB1, TFB2M, TNS3
5	11	0.0748	0.4021	BAZ2A, DDAH2, DNMT1, EID2, FKBP3, HDAC2, PADI4, SAP30, TAF6, TBC1D8, WDTC1
6	9	0.0612	0.5257	CD40LG, DUSP12, DUSP14, DUSP3, EIF2AK3, KMT2C, MAP3K4, MAPK14, MAPKAPK5
7	9	0.0612	0.4102	ACTA2, ITGA2B, ITGB5, MYBPC3, MYL9, SORBS3, TLN1, TPM1, TPM2
8	8	0.0544	0.6486	CPSF2, HNRNPA0, HNRNPH1, HNRNPU, SNRPA1, SRSF2, SRSF7, ZRANB2
9	8	0.0544	0.5985	AKR1B1, CFLAR, CTDSPL, DROSHA, SMAD3, TFE3, TGIF2, ZMYND11
10	8	0.0544	0.4305	AK3, CDK5RAP2, CEP78, OFD1, TUBA4A, TUBB4B, TUBGCP5, VBP1
11	7	0.0476	0.4346	CARD16, DNAJC10, ERLEC1, HSPE1, SLX4, USP10, VCP
12	7	0.0476	0.3656	FES, FGF9, GSN, PELP1, PIK3R1, PIK3R5, PXK

Cluster 1 was enriched by most of the ribosomal protein genes, and cluster 2 was enriched by *NUP160* and *HNRNPU*. The ribosome pathway and the spliceosome pathway were significantly enriched in cluster 1 and cluster 2, respectively.

In this study, modules and clusters had similar meanings. The "modules" were obtained using ReactomeFI, and the "clusters" were obtained by ClusterOne. Sometimes, the results obtained by ClusterOne also could be called "modules". The main difference between modules and clusters was that the former was function modules and the latter was cluster modules. In the present study, the results obtained by ClusterOne were presented as "clusters".

Discussion

With the gene expression patterns obtained from the GEO database, a total of 630 upregulated and 1,002 downregulated genes were differently expressed in PDAC samples compared with healthy samples in our present study. Our results showed some ribosomal protein genes with higher average correlation in module 0 were enriched in the ribosome pathway. *NUP107* and *NUP160* were enriched in module 3. *HNRNPU,* with higher

average correlation in module 8, was enriched in the spliceosome pathway. The ribosome pathway and the spliceosome pathway were significantly enriched in cluster 1 and cluster 2, respectively.

RPS6 is an mTOR effector and the mTOR pathway is activated in PDAC [26,27]. Phosphorylation of *RPS6* attenuates DNA damage and tumor suppression for pancreatic cancer progression [26]. *RPS26* regulates *p53* activity and *p53* plays an important role in the development of PDAC [28,29]. Furthermore, the abundance of *RPS8* can determine the susceptibility of pancreatic cancer cells to gemcitabine treatment [30]. In addition, a higher abundance of *RPS8* is related to worse survival of PDAC, and *RPS8* may be an important prognostic factor [31]. In addition, downregulation of *RPL15* is related to tumor development in PDAC [32]. Thus, some ribosomal protein genes, such as *RPS6*, *RPS26* and *RPS8*, have been associated with the progression of PDAC. In the present study, some ribosomal protein genes with higher average correlation in module 0 were also enriched in cluster 1. Although the significant roles of some other ribosomal protein genes have not been fully discussed, we speculated that ribosomal protein genes may be involved in the development of PDAC.

Table 2. Pathways significantly enriched by each module.

| Module	GeneSet	FDR	Nodes
0	SRP-dependent cotranslational protein targeting to membrane(R)	<1.667e-04	RPL17, RPL36A, RPL13, RPL37, RPL39, RPL7, RPS3A, RPL31, RPS23, RPL35A, SEC11C, RPL26, SRPRB, RPS6, RPS7, RPL23, RPS17, RPL22, RPL21, SEC61G, SRP9
0	Eukaryotic Translation Termination(R)	<1.667e-04	RPL17, RPL36A, RPL13, RPL37, RPL39, RPL7, RPS3A, RPL31, RPS23, RPL35A, RPL26, RPS6, RPS7, RPL23, RPS17, RPL22, RPL21
0	Ribosome(K)	<1.667e-04	RPL17, RPL36A, RPL13, RPL37, RPL39, RPL7, RPS3A, RPL31, RPS23, RPL35A, RPL26, RPS6, RPS7, RPL23, RPS17, RPL22, RPL21
2	Mitochondrial translation(R)	<1.000e-03	MRPS35, MRPS17, MRPS26, MRPL1, MRPS33, MRPL50, MRPL3, MRPS23, MRPS30, MRPL24, MRPL12, ICT1, MRPL38, MRPL39, MRPL48
2	Ribosome(K)	<5.000e-04	MRPS17, MRPL1, MRPL3, MRPL24, MRPL12
3	ISG15 antiviral mechanism(R)	<1.000e-03	NUP160, NUP88, NUP205, NUP107, NUP54, KPNA3
3	Hexose transport(R)	<5.000e-04	NUP160, NUP88, NUP205, NUP107, NUP54
3	Metabolism of non-coding RNA(R)	<3.333e-04	NUP160, NUP88, NUP205, NUP107, NUP54
5	NoRC negatively regulates rRNA expression(R)	<1.000e-03	SAP30, HDAC2, DNMT1, BAZ2A
5	Signaling events mediated by HDAC Class I(N)	<5.000e-04	SAP30, HDAC2, FKBP3
5	Hedgehog signaling events mediated by Gli proteins(N)	3.83e-02	SAP30, HDAC2
6	Oxidative stress response(P)	<1.000e-03	DUSP3, MAP3K4, DUSP14, MAPK14, MAPKAPK5, DUSP12
6	MAPK signaling pathway(K)	8.00e-03	DUSP3, MAP3K4, MAPK14, MAPKAPK5
6	p38 MAPK signaling pathway(N)	2.17e-02	MAP3K4, MAPK14
7	Muscle contraction(R)	<1.000e-03	SORBS3, TLN1, ACTA2, MYBPC3, ITGB5, TPM2, TPM1, MYL9
7	Hypertrophic cardiomyopathy (HCM)(K)	<5.000e-04	MYBPC3, ITGB5, TPM2, TPM1, ITGA2B
7	Dilated cardiomyopathy(K)	<3.333e-04	MYBPC3, ITGB5, TPM2, TPM1, ITGA2B
8	Processing of Capped Intron-Containing Pre-mRNA(R)	<1.000e-03	SRSF2, SNRPA1, SRSF7, HNRNPH1, CPSF2, HNRNPA0, HNRNPU
8	Spliceosome(K)	<5.000e-04	SRSF2, SNRPA1, SRSF7, HNRNPU
8	spliceosomal assembly(B)	1.33e-03	SRSF2, SNRPA1
9	Regulation of nuclear SMAD2/3 signaling(N)	<1.000e-03	TFE3, SMAD3, TGIF2
9	Regulation of cytoplasmic and nuclear SMAD2/3 signaling(N)	7.00e-03	CTDSPL, SMAD3
10	Mitotic G2-G2/M phases(R)	<1.000e-03	OFD1, TUBGCP5, CEP78, TUBA4A, CDK5RAP2, TUBB4B
10	Assembly of the primary cilium(R)	<5.000e-04	OFD1, CEP78, TUBA4A, CDK5RAP2, TUBB4B
10	Protein folding(R)	<3.333e-04	VBP1, TUBA4A, TUBB4B
11	Protein processing in endoplasmic reticulum(K)	2.00e-03	VCP, DNAJC10, ERLEC1
11	Hedgehog ligand biogenesis(R)	2.15e-02	VCP, ERLEC1
12	Signaling by SCF-KIT(R)	7.00e-03	FGF9, FES, PIK3R1
12	Nongenotropic Androgen signaling(N)	9.00e-03	PELP1, PIK3R1
12	Osteopontin-mediated events(N)	7.67e-03	GSN, PIK3R1

FDR – false discovery rate.
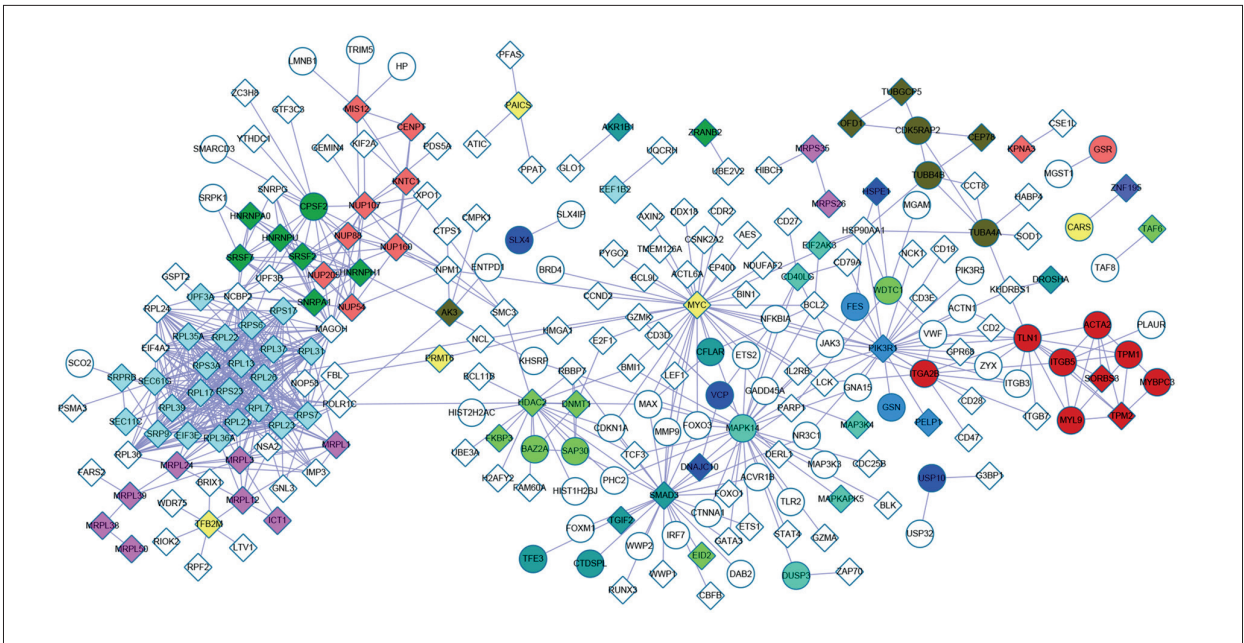
Figure 3. The PPI network of other differentially expressed genes (DEGs) interacted with 13 module genes. White represents other DEGs; the color of the modules was in line with Figure 2.
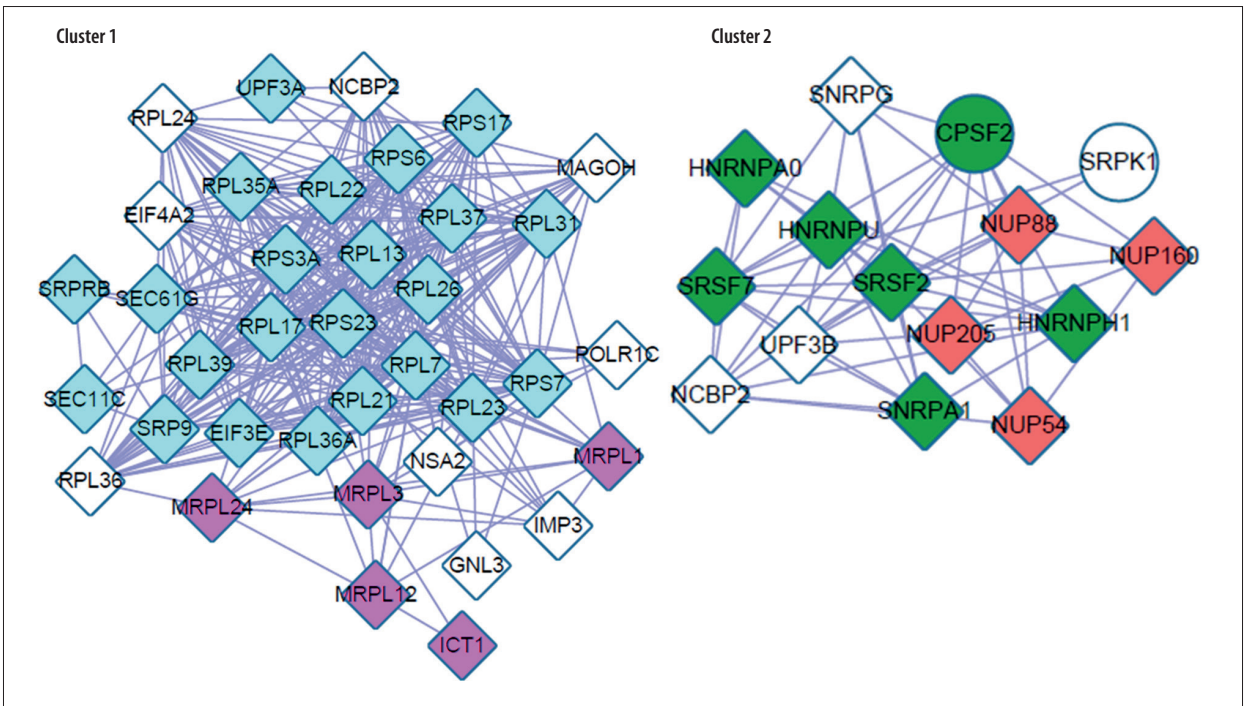

Figure 4. Two significantly enriched subnetwork modules (cluster 1 and cluster 2).

Nup170 and *Nup160* are significant components of the Nup107-160 complex, and the Nup107-160 complex plays an important role in the regulation of NPC (nuclear pore complex) assembly [33]. On the one hand, the Nup107-160 complex regulates microtubule polymerization at kinetochores [34]. Nup107-160 complex is an essential component for the recruitment

of RanGAP1 (Ran GTPase-activating protein 1), RanBP2 (Ran GTPase-activating protein 2), and Crm1 (chromosomal maintenance 1) to these structures at kinetochores [35]. Furthermore, *Nup170* is involved in kinetochore function and the overexpression of kinetochore may contribute to the development of tumor via driving chromosome instability [36,37]. On the other
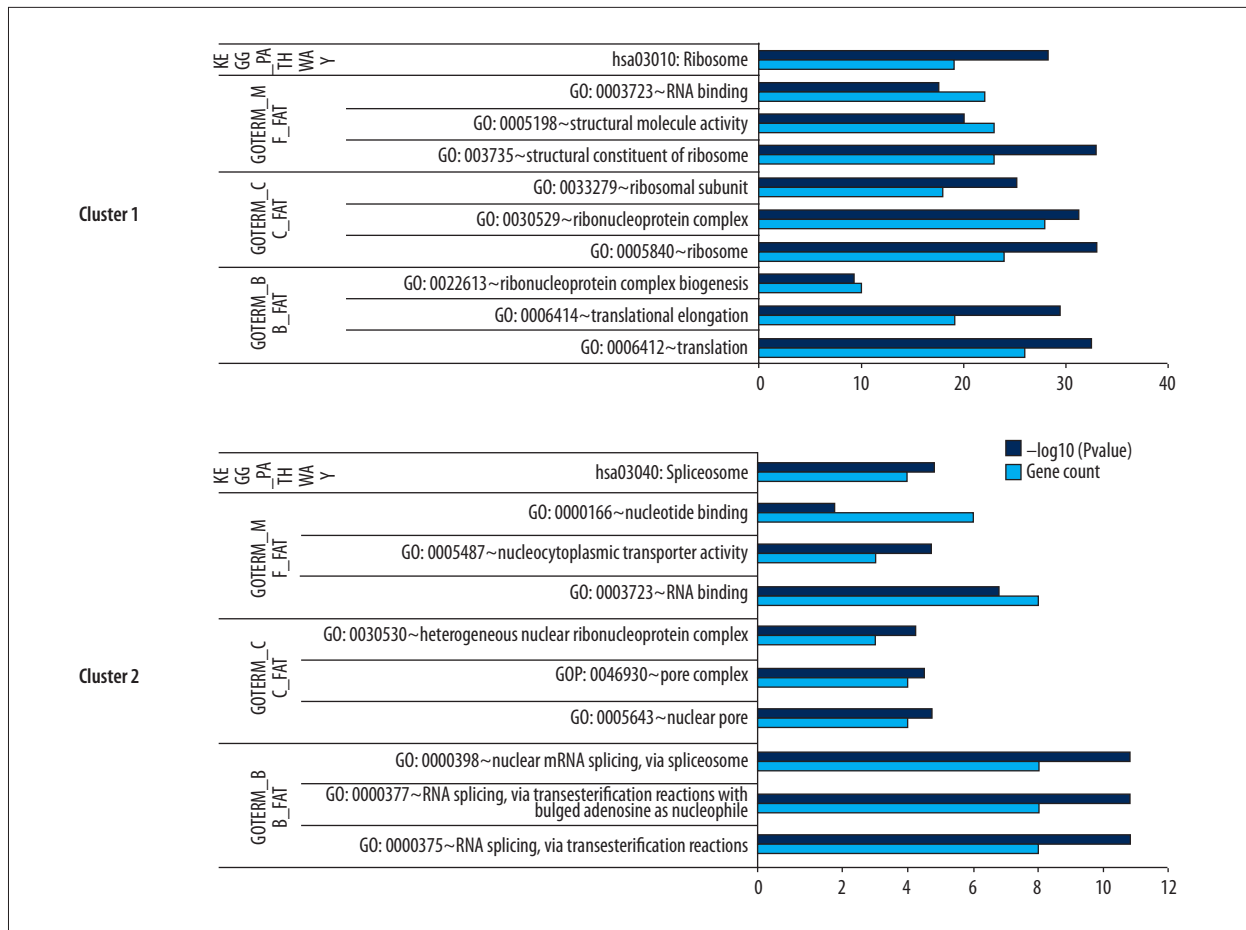
Figure 5. GO and KEGG pathways significantly enriched by two subnetwork modules.

hand, Ran is an essential protein in the formation of nuclear envelope, and this mechanism is dependent on importin-β associated with Nup107-Nup160 [38]. Furthermore, Ran plays a significant role in the metastatic progression of cancer [38]. One study indicated that *Nup170* was upregulated in pancreatic cancer [33]. In our present study, *NUP107* and *NUP160* were enriched in module 3, and *NUP160* also was enriched in cluster 2. Thus, we speculated that *Nup170* and *Nup160* may play important roles in the development of PDAC.

HNRNPU can modulate *WT1* transcriptional activation by directly interacting with *WT1* [39], and *WT1* plays a significant part in the tumorigenesis of PDAC [11]. *HNRNPU* interacts with *MDM2* (E3 ubiquitin-protein ligase Mdm2) involving the degradation of *p53* [40]. Furthermore, *p53* as tumor suppressor genes plays a part in cell cycle regulation, and mutation of this gene may promote the growth of tumor cell [41]. Thus, *HNRNPU* may play a role in PDAC progression indirectly. In this study, *HNRNPU* with higher average correlation in module 8 was enriched in cluster 2. Therefore, our results were similar to other research and suggest that *HNRNPU* may be an important gene involved in the development of PDAC.

One study indicated that *Myc* might be involved in the early neoplastic progression of PDAC [42]. Furthermore, the spliceosome is a treatment entry point for *Myc*-driven cancer [43]. Thus, spliceosome may play important roles in the PDAC progression. In this study, the spliceosome pathway was significantly enriched in cluster 2, and *HNRNPU* was enriched in the spliceosome pathway. Therefore, *HNRNPU* may play important roles in the development of PDAC via the spliceosome pathway. In addition, the ribosome pathway was significantly enriched in cluster 1, and the ribosomal protein genes mainly enriched in the ribosome pathway. In addition, as aforementioned, the ribosomal protein genes may be involved in the development of PDAC. Therefore, ribosomal protein genes may play significant parts in the development of PDAC via ribosome pathway.

Conclusions

Ribosomal protein genes, *Nup170*, *Nup160*, *HNRNPU*, and the ribosome pathway, as well as the spliceosome pathway, may play important roles in PDAC progression. In addition, ribosomal protein genes, *Nup170*, *Nup160*, and *HNRNPU* may be

used as possible molecular markers for the early diagnosis of the disease. A limitation of this study is a lack of experimental verification, thus, further research is needed about genes that are used for early diagnosis of the disease and other possible molecular markers of PDAC.

Conflict of interest

None.

References:

1. Li D, Xie K, Wolff R, Abbruzzese JL: Pancreatic cancer. Lancet, 2004; 363(9414): 1049–57

2. Ying H, Dey P, Yao W et al: Genetics and biology of pancreatic ductal adenocarcinoma. Genes Dev, 2016; 30(4): 355–85

3. Siegel R, Naishadham D, Jemal A: Cancer statistics, 2013. Cancer J Clin, 2013; 63(1): 11–30

4. Bond-Smith G, Banga N, Hammond TM, Imber CJ: Pancreatic adenocarcinoma. BMJ, 2012; 344: e2476

5. Stewart B, Wild C: World cancer report 2014: World Health Organization. Geneva; 2014

6. Szafranska A, Davison T, John J et al: MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma. Oncogene, 2007; 26(30): 4442–52

7. Liu Q, Chen J, Wang J et al: Putative tumor suppressor gene SEL1L was downregulated by aberrantly upregulated hsa-mir-155 in human pancreatic ductal adenocarcinoma. Mol Carcinog, 2014; 53(9): 711–21

8. Steele CW, Oien KA, McKay CJ, Jamieson NB: Clinical potential of microRNAs in pancreatic ductal adenocarcinoma. Pancreas, 2011; 40(8): 1165–71

9. Frampton AE, Krell J, Jacob J et al: microRNAs as markers of survival and chemoresistance in pancreatic ductal adenocarcinoma. Exp Rev Anticancer Ther, 2011; 11(12): 1837–42

10. Fukuda A, Wang SC, Morris JP et al: Stat3 and MMP7 contribute to pancreatic ductal adenocarcinoma initiation and progression. Cancer Cell, 2011; 19(4): 441–55

11. Oji Y, Nakamori S, Fujikawa M et al: Overexpression of the Wilms' tumor gene WT1 in pancreatic ductal adenocarcinoma. Cancer Sci, 2004; 95(7): 583–87

12. Morton JP, Timpson P, Karim SA et al: Mutant p53 drives metastasis and overcomes growth arrest/senescence in pancreatic cancer. Proc Natl Acad Sci, 2010; 107(1): 246–51

13. Xia J-T, Wang H, Liang L-J et al: Overexpression of FOXM1 is associated with poor prognosis and clinicopathologic stage of pancreatic ductal adenocarcinoma. Pancreas, 2012; 41(4): 629–35

14. Denley SM, Jamieson NB, McCall P et al: Activation of the IL-6R/Jak/stat pathway is associated with a poor outcome in resected pancreatic ductal adenocarcinoma. J Gastrointest Surg, 2013; 17(5): 887–98

15. Pai P, Rachagani S, Lakshmanan I et al: The canonical Wnt pathway regulates the metastasis-promoting mucin MUC4 in pancreatic ductal adenocarcinoma. Mol Oncol, 2016; 10(2): 224–39

16. Iacobuzio-Donahue CA, Ashfaq R, Maitra A et al: Highly expressed genes in pancreatic ductal adenocarcinomas A comprehensive characterization and comparison of the transcription profiles obtained from three major technologies. Cancer Res, 2003; 63(24): 8614–22

17. Smyth GK: LIMMA: Linear models for microarray data. In: Gentleman R, Carey VJ, Huber W et al. (eds.): Bioinformatics and Computational Biology Solutions Using R and Bioconductor. Statistics for Biology and Health. Springer, New York, NY, 2005; 397–420

18. Dunning M, Lynch A, Eldridge M: IlluminaHumanv4. db: Illumina HumanHT12v4 annotation data (chip illuminaHumanv4). R package version 2013; 2(0)

19. Berkeley C: Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. E-book available at *http://www bepress com/sagmb/vol3/iss1/art3* 2004

20. Ferreira JA: The Benjamini-Hochberg method in the case of discrete test statistics. Int J Biostat, 2007; 3(1): Article 11

21. Wu G, Dawson E, Duong A et al: ReactomeFIViz: A Cytoscape app for pathway and network-based data analysis. Version 2. F1000Res, 2014 [revised 2014 Sep 12]; 3:146

22. Shannon P, Markiel A, Ozier O et al: Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res, 2003; 13(11): 2498–504

23. Szklarczyk D, Franceschini A, Wyder S et al: STRING v10: Protein–protein interaction networks, integrated over the tree of life. Nucleic Acids Res, 2015; 43(Database issue): D447–52

24. Kanehisa M, Goto S: KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res, 2000; 28(1): 27–30

25. Nepusz T, Yu H, Paccanaro A: Detecting overlapping protein complexes in protein-protein interaction networks. Nat Methods, 2012; 9(5): 471–72

26. Khalaileh A, Dreazen A, Khatib A et al: Phosphorylation of ribosomal protein S6 attenuates DNA damage and tumor suppression during development of pancreatic cancer. Cancer Res, 2013; 73(6): 1811–20

27. Bellizzi AM, Bloomston M, Zhou X-P et al: The mTOR pathway is frequently activated in pancreatic ductal adenocarcinoma and chronic pancreatitis. Appl Immunohistochem Mol Morphol, 2010; 18(5): 442–47

28. Navas C, Hernández-Porras I, Schuhmacher AJ et al: EGF receptor signaling is essential for k-ras oncogene-driven pancreatic ductal adenocarcinoma. Cancer Cell, 2012; 22(3): 318–30

29. Cui D, Li L, Lou H et al:The ribosomal protein S26 regulates p53 activity in response to DNA damage. Oncogene, 2014; 33(17): 2225–35

30. Toshimitsu H, Iizuka N, Yamamoto K et al: Molecular features linked to the growth-inhibitory effects of gemcitabine on human pancreatic cancer cells. Oncol Rep, 2006; 16(6): 1285–91

31. Chen R, Dawson DW, Pan S et al: Proteins associated with pancreatic cancer survival in patients with resectable pancreatic ductal adenocarcinoma. Lab Invest, 2015; 95(1): 43–55

32. Yan T-T, Fu X-L, Li J et al: Downregulation of RPL15 may predict poor survival and associate with tumor progression in pancreatic ductal adenocarcinoma. Oncotarget, 2015; 6(35): 37028–42

33. Tan X, Zhou L, Wang W et al: Genomic analysis of invasion-metastasis-related factors in pancreatic cancer cells. Exp Ther Med, 2010; 1(1): 211–16

34. Mishra RK, Chakraborty P, Arnaoutov A et al: The Nup107-160 complex and γ-TuRC regulate microtubule polymerization at kinetochores. Nat Cell Biol, 2010; 12(2): 164–69

35. Zuccolo M, Alves A, Galy V et al: The human Nup107–160 nuclear pore subcomplex contributes to proper kinetochore functions. EMBO J, 2007; 26(7): 1853–64

36. Flemming D, Sarges P, Stelter P et al: Two structurally distinct domains of the nucleoporin Nup170 cooperate to tether a subset of nucleoporins to nuclear pores. J Cell Biol, 2009; 185(3): 387–95

37. Yuen KW, Montpetit B, Hieter P: The kinetochore and cancer: What's the connection? Curr Opin Cell Biol, 2005; 17(6): 576–82

38. Matchett KB, McFarlane S, Hamilton SE et al: Ran GTPase in nuclear envelope formation and cancer metastasis. Adv Exp Med Biol, 2014; 773: 323–51

39. Spraggon L, Dudnakova T, Slight J et al: hnRNP-U directly interacts with WT1 and modulates WT1 transcriptional activation. Oncogene, 2007; 26(10): 1484–91

40. Hegde ML, Banerjee S, Hegde PM et al: Enhancement of NEIL1 protein-initiated oxidized DNA base excision repair by heterogeneous nuclear ribonucleoprotein U (hnRNP-U) via direct interaction. J Biol Chem, 2012; 287(41): 34202–11

41. Wagenhuser M, Rockert F, Niedergethmann M et al: Distribution of characteristic mutations in native ductal adenocarcinoma of the pancreas and pancreatic cancer cell lines. Cell Biol: Res Ther, 2013; 2: 1

42. Schleger C, Verbeke C, Hildenbrand R et al: c-MYC activation in primary and metastatic ductal adenocarcinoma of the pancreas: Incidence, mechanisms, and clinical significance. Mod Pathol, 2002; 15(4): 462–69

43. Hsu TY-T, Simon LM, Neill NJ et al: The spliceosome is a therapeutic vulnerability in MYC-driven cancer. Nature, 2015; 525(7569): 384–88