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Identification of key pathways and genes changes in pancreatic cancer cells (BXPC-3) after cross-talk with primary pancreatic stellate cells using bioinformatics analysis

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most malignant tumors with poor prognosis, and the interaction between activated pancreatic stellate cells (PSCs) and PDAC cells plays an important role in the development of PDAC. The aim of this study was to identify gene changes in BXPC-3 after cross-talk with PSCs and reveal their potential mechanisms. The gene expression profiling analysis of BXPC-3 was completed after co-culture with primary PSCs for 48 h. The gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes pathway (KEGG) enrichment analyses were performed, and the differentially expressed genes (DEGs) were identified by Agilent GeneSpring GX software. In total, 3657 DEGs were identified in BXPC-3, including 1881 up-regulated genes and 1776 downregulated genes. GO analysis results showed that upregulated DEGs were significantly enriched in biological processes (BP), including peptide metabolic process, response to stress and electron transport chain; the downregulated DEGs were significantly enriched in biological processes, including signaling, multicellular organism development and anatomical structure development. KEGG pathway analysis revealed that 19 pathways were upregulated and 32 pathways were downregulated, and that upregulated DEGs were enriched in protein export and glutathione metabolism, while the downregulated DEGs were enriched in axon guidance and focal adhesion. The top 10 upregulated genes and the top 10 downregulated genes were identified. By constructing PPI network, we selected out 10 key genes (TP53, SRC, IL6, JUN, ISG15, CAD, STAT1, OAS3, OAS1, VIM) and significant pathways. The associated survival analysis was performed and the SRC, IL-6, ISG15, STAT1, OAS3, OAS1 and VIM were proved to be related to worse overall survival time of PDAC patients. In conclusion, the present study indicated that the identified DEGs promote our understanding of the molecular mechanisms underlying the interaction between pancreatic cancer cells and PSCs and might be used as molecular targets in the future to study the role of tumor microenvironment in the progression of PDAC.

Key words: bioinformatics analysis, pancreatic duct adenocarcinoma, pancreatic stellate cells, different expression genes

Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal disease and is the fourth leading cause of cancer death, with a median survival time of approximately 6 months and a 5-year survival rate of less than 5% [1]. At diagnosis, 50–60% of patients have advanced disease with distant metastases and of the 10% or so of patients who undergo a curative resection, many will relapse with distant metastases and/or locoregionally [2, 3]. The poor prognosis of PDAC is due to its aggressive growth and rapid development of distant metastases, low rate of eligibility for surgical resection and chemoradiation resistance, thus making treatment extremely difficult [4]. Over recent 20 years, activated pancreatic stellate cells (PSCs) were identified in the desmoplastic microenvironment of PDAC and acted as an active player in carcinogenesis [4, 5]. Increasing evidence indicates that the interaction between activated PSCs and PDAC cells plays an important role in the development of PDAC. By producing high levels of cytokines, chemotactic factors, growth factors and excessive ECM, PSCs create desmoplasia and a hypoxic microenviron-

ment that promote the initiation, development, evasion of immune surveillance, invasion, metastasis and resistance to chemoradiation of PDAC [6–11]. Therefore, targeting the interaction between PSCs and PDAC cells may represent a novel therapeutic approach to advanced PDAC [4, 5, 12]. Accumulating evidence also has demonstrated that multiple genes and cellular pathways participate in the interaction between activated PSCs and PDAC cells [13, 14]. However, despite increased interest in this microenvironment, there is lack of critical understanding of knowledge regarding the precise molecular mechanisms and specific roles underlying the cross-talks between PSCs and PDAC cells which limits the ability to treat the advanced disease. Therefore, understanding the molecular mechanism involved in the interaction between PSCs and PDAC cells is extremely important for the development of more effective diagnostic and therapeutic strategies for PDAC.

Activated PSCs have a profound influence on the development and dissemination of PDAC, and play a key role in this tumor microenvironment not only tumor promoting but also protective [15]. Previous studies on PSCs have attributed their interaction roles with PDAC cells to the regulation of many signal transduction pathways, including HGF-c-MET pathway [16], IL-6/STAT3 pathway that regulates the PSC-induced EMT and alters gene expression in pancreatic cancer cells [17], SDF-1alpha/CXCR4 signaling that can be therapeutic target to overcome chemoresistance in pancreatic cancer [18], and Wnt/beta-catenin signaling pathway that can promote the progression of PDAC [19]. PSCs have shown changed gene expression profile and confer more malignant biology behavior on the pancreatic cancer cells [20–22]. The different expression genes (DEGs) and the special signal pathways of the interaction between PSCs and PCCs in independent studies show a relatively limited degree of overlap, and no reliable biomarker profile discriminating the action of PSCs on PCCs (or not) has been identified. Therefore, the interactions among DEGs, particularly the pathways in the interaction network between PSCs and PCCs, remain to be elucidated.

In the present study, we performed the gene expression profiling analysis of BXPC-3 co-cultured with or without primary PSCs. Subsequently, the DEGs were screened using Gene-Spring software, followed by gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and protein–protein interaction (PPI) network analysis. We therefore performed a global analysis of PSCs-fostered signaling pathways and related genes in PCCs on the basis of gene expression profile and bioinformatic interpretation. By way of analyzing their biological functions and pathways, we may gain further insight on PDAC development at molecular level and explore the potential candidate biomarkers for diagnosis, prognosis and drug targets. The predicted signaling pathways, some of which had not been previously described in the interaction between PSCs and PCCs, were worthy of further selection and validation.

Materials and methods

Cells, culture conditions, RNA extraction and quantitative reverse transcription-polymerase chain reaction. The primary human PSCs isolation, identification and maintenance were described previously [11, 23, 24]. PSCs from passage numbers 2 to 5 were used for all assays. Pancreatic cancer cell line (BxPC-3) was grown in DMEM with 10% FBS. Co-culture experiments were performed as follows: monolayers of primary PSCs (1×10^5 cells) co-cultured with BxPC-3 cells (5×10^5 cells) in upper and lower part of the transwell (six wells, 0.4 μm pores provided by Corning Co) at 37 °C for 48 h, separately. After co-culture, the BxPC-3 cells were then harvested. Total RNA was extracted from cultured cells using Trizol reagent (Invitrogen, Beijing, China) according to the manufacturer's instructions. RNA quantity and quality were measured by NanoDrop ND-1000. RNA integrity was assessed by standard denaturing agarose gel electrophoresis. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed as previously described [11, 23, 24].

DNA microarray. The human 12 x 135K Gene Expression Array was manufactured by Roche NimbleGen. About 45033 genes were collected from the authoritative data source including NCBI.

RNA labeling and array hybridization. Double-strand cDNA (ds-cDNA) was synthesized from total RNA using an Invitrogen SuperScript ds-cDNA synthesis kit in the presence of 100 pmol oligo dT primers. ds-cDNA was cleaned and labeled in accordance with the NimbleGen Gene Expression Analysis protocol (NimbleGen Systems, Inc., Madison, WI, USA). The labeled ds-cDNA was purified by isopropanol/ethanol precipitation. Microarrays were hybridized at 42 °C during 16 to 20 h with 4 μg of Cy3-labeled ds-cDNA in a hybridization chamber (Hybridization System – NimbleGen Systems, Inc., Madison, WI, USA), and then the slides were scanned using the Axon GenePix 4000B microarray scanner.

Data collection and identification of DEGs. Slides were scanned at 5 μm/pixel resolution using an Axon GenePix 4000B scanner (Molecular Devices Corporation) piloted by GenePix Pro 6.0 software (Axon). Scanned images (tiff format) were then imported into NimbleScan software (version 2.5) for grid alignment and expression data analysis. Expression data were normalized through quantile normalization and the Robust Multichip Average (RMA) algorithm included in the NimbleScan software. All gene level files were imported into Agilent GeneSpring GX software (version 11.5.1) for further analysis. Differentially expressed genes (DEGs) were identified through Fold Change filtering and we used a classical t test to identify DEGs with a change ≥ two-fold and defined a p-value cutoff of <0.05 to be statistically significant. Hierarchical clustering was performed using the Agilent GeneSpring GX software (version 11.5.1). GO analysis and Pathway analysis were performed using the standard enrichment computation method.

Gene ontology and pathway enrichment analysis of DEGs. Gene ontology analysis (GO) (http://www.geneontology.org) was applied in order to organize genes into hierarchical categories and uncover the gene regulatory network on the basis of biological process and molecular function [25]. KEGG (http://www.genome.jp) is a knowledge base for systematic analysis of gene functions, linking genomic information with higher-order functional information [26]. Comprehensively, mapping of user's gene to the relevant biological annotation in the DAVID database (https://david.ncifcrf.gov) is an essential foundation for the success of any high-throughput gene functional analysis [27]. In order to analyze the DEGs at the functional level, GO enrichment and KEGG pathway analysis were performed using DAVID online tool. In detail, a two-sided Fisher's exact test and chi-square test were used to classify the enrichment of pathway category, and the false discovery rate (FDR) was calculated to correct the p-value. We chose only pathways that had a p-value of <0.01 and an FDR of <0.01.

Integration of protein–protein interaction network and module analysis. Search Tool for the Retrieval of Interacting Genes (STRING version 10.5) database is online tool designed to evaluate the protein–protein interaction (PPI) information [28]. We mapped the DEGs to STRING to evaluate the relationships and potential functions among the DEGs. We set combined score >0.4 as significant and the selected data was introduced to open-source Cytoscape software. This powerful tool can visualize complex PPI networks and perform relative analysis (http://cytoscape.org/) [29]. The plug-in MCODE and CytoNCA were used to screen the modules of PPI network and explore key genes in the PPI network. In addition, we carried out function and pathway enrichment analysis for the modules.

The survival analysis of hub genes. The Kaplan-Meier plotter (KM plotter, http://kmplot.com/ analysis) was applied to evaluate the prognosis of patients with PDAC. We analyzed the effects of hub genes with high or low levels of mRNA expression on patients' overall survival (OS). The

Table 1. Top 10 upregulated and downregulated DEGs in BXPC-3 after cross-talked with primary PSCs.

SEQ_ID	GENE ID	GENE NAME	DESCRIPTION	GO biological process
Top 10 upregulated genes				
NM_022147	64108	RTP4	receptor transporter protein 4	GO:0006612(protein targeting to membrane)
NM_000804	2352	FOLR3	folate receptor 3 (gamma)	GO:0015884(folic acid transport)
NM_001017364	725	C4BPB	complement component 4 binding protein, beta	GO:0045087(innate immune response)
AY495087	5138	PDE2A	phosphodiesterase 2A, cGMP-stimulated	GO:0007165(signal transduction)
NM_022805	6638	SNRPN	small nuclear ribonucleoprotein polypeptide N	GO:0016071(mRNA metabolic process)
BC017784	8302	KLRC4	killer cell lectin-like receptor subfamily C, member 4	GO:0006968(cellular defense response)
NM_032843	84929	FIBCD1	fibrinogen C domain containing 1	GO:0007165(signal transduction)
NM_016730	2348	FOLR1	folate receptor 1 (adult)	GO:0006898(receptor-mediated endocytosis)
NM_001846	1284	COL4A2	collagen, type IV, alpha 2	GO:0030198(extracellular matrix organization and biogenesis)
NM_006744	5950	RBP4	retinol binding protein 4, plasma	GO:0006810(transport)
Top 10 downregulated genes				
NM_004387	1482	NKX2-5	NK2 transcription factor related, locus 5 (Drosophila)	GO:0000122(negative regulation of transcription from RNA polymerase II promoter);
BC018096	8153	RND2	Rho family GTPase 2	GO:0007264(small GTPase mediated signal transduction)
NM_020404	57124	CD248	CD248 molecule, endosialin	GO:0008150(biological_process)
BC062415	4239	MFAP4	microfibrillar-associated protein 4	GO:0007155(cell adhesion)
NM_000474	7291	TWIST1	twist homolog 1 (acrocephalosyndactyly 3; Saethre-Chotzen syndrome) (Drosophila)	GO:0000122(negative regulation of transcription from RNA polymerase II promoter)
AK127541	165	AEBP1	AE binding protein 1	GO:0007155(cell adhesion)
NM_002615	5176	SERPINF1	serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	GO:0008283(cell proliferation)
NM_001014434	56956	LHX9	LIM homeobox 9	GO:0006355(regulation of transcription, DNA-dependent)
BC028170	4081	MAB21L1	mab-21-like 1 (C. elegans)	GO:0009653(anatomical structure morphogenesis)
NM_032498	84528	PEPP-2	PEPP subfamily gene 2	GO:0006355(regulation of transcription, DNA-dependent)

SEQ_ID: NCBI Reference Sequence ID; GENE_ID: GENE_ID in NCBI.

hazard ratio (HR) with 95% confidence intervals and log rank p-values were calculated.

Results

Morphology of co-culture system and identification of DEGs. The immunohistochemical staining results showed the morphology of pure PSCs, pure BXPC-3 and BXPC-3 and PSCs co-culture system (Figure S1). The series from each chip were analyzed separately using GeneSpring software and finally identified the DEGs lists. Based on the GeneSpring analysis, using $p<0.05$ and fold control (FC) ≥ 2.0 criteria, a total of 3657 genes were identified, of which 1881 were upregulated and 1776 were downregulated. The top 10 upregulated genes, RTP4, FOLR3, C4BPB, PDE2A, SNRPN, KLRC4, FIBCD1, FOLR1, COL4A2 and RBP4, and the top 10 downregulated genes, NKX2-5, RND2, CD248, MFAP4, TWIST1, AEBP1, SERPINF1, LHX9, MAB21L1 and PEPP-2 were identified as well (Table 1). The results of qRT-PCR were consistent with the sequencing results (Figure S2). The boxplot, scatterplot and DEGs expression heat map is shown in Figure 1.

GO term enrichment analysis. We uploaded all DEGs to the online software DAVID to identify overrepresented GO categories and KEGG pathways. GO analysis results showed that upregulated DEGs were significantly enriched in biological processes (BP), including peptide metabolic process, response to stress and electron transport chain; the downregulated DEGs were significantly enriched in biological processes, including signaling, multicellular organismal development and anatomical structure development (Table S1). For molecular function (MF), the upregulated DEGs were enriched in protein binding, glutathione transferase activity and ATPase activity, and the down-regulated DEGs were enriched in protein binding, protein kinase activity and phosphotransferase activity (Table S1). In addition, GO cell component (CC) analysis also displayed that the upregulated DEGs were significantly enriched in cytoplasm, intracellular and intracellular part, and downregulated DEGs were enriched in plasma membrane part, caveola and plasma membrane (Table S1). In addition, the top ten counts of the significant enrichment terms and the p-value trees were also showed in the Figures S3a and S3b.

KEGG pathway analysis. Table S2 contains the most significantly enriched pathways of the upregulated DEGs and downregulated DEGs analyzed by KEGG analysis, in which 19 signal transduction pathways were upregulated and 32 were downregulated. The upregulated DEGs were enriched in protein export and glutathione metabolism, while the downregulated DEGs were enriched in axon guidance, focal adhesion, basal cell carcinoma and MAPK signaling pathway (Figure 2).

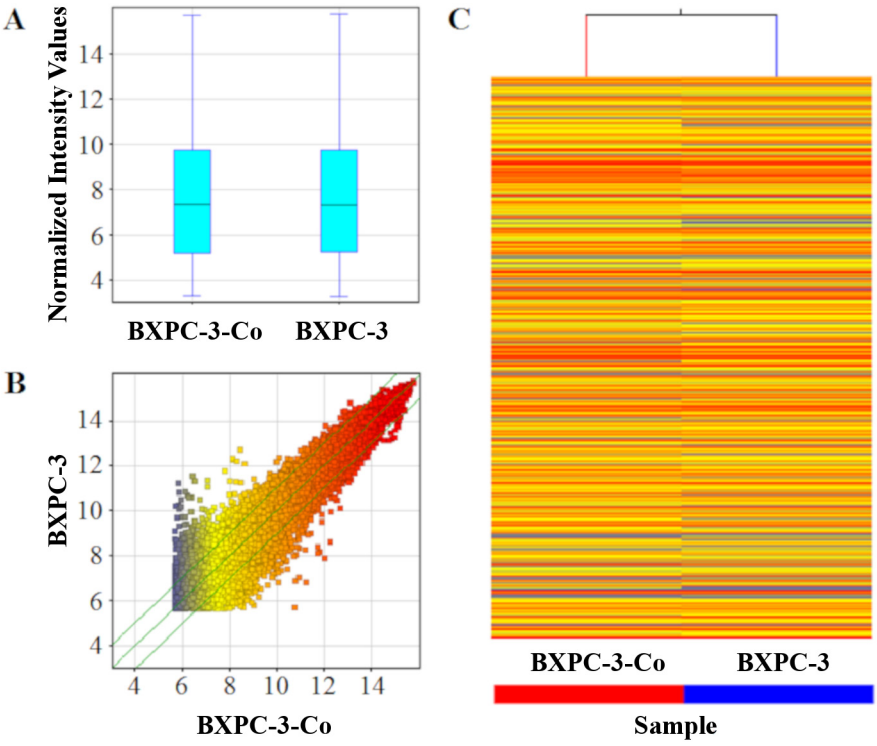


Figure 1. A) Boxplot view is used to look at and compare the distributions of expression values for the samples in an experiment after normalization. B) Scatterplot is a visualization that is useful for assessing the variation (or reproducibility) between chips. C) Heat map and hierarchical clustering. Hierarchical Clustering for "All Targets Value". "Red" indicates high relative expression, and "blue" indicates low relative expression.

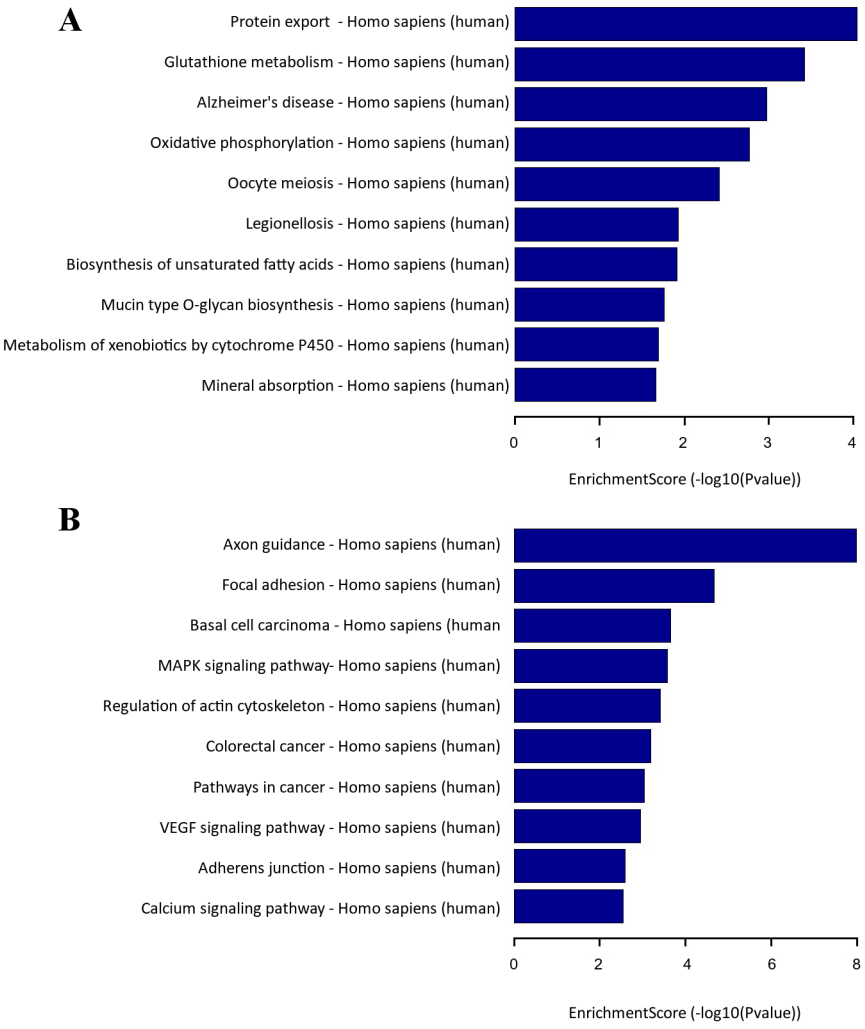


Figure 2. A) Top 10 upregulated hsa_EnrichmentScore; B) Top 10 downregulated hsa_EnrichmentScore.

Key nodes and modules of the PPI network. Based on the STRING database, the DEGs with the highest PPI scores identified by the three centrality methods are shown in Table 2. We selected out 10 key genes based on the information of the PPI network, including TP53 (tumor protein p53), SRC (SRC proto-oncogene), IL-6 (interleukin 6), JUN (Jun proto-oncogene), ISG15 (Interferon-stimulated gene 15 kDa), CAD (carbamoyl-phosphate synthetase 2), STAT1 (signal transducer and activator of transcription 1), OAS3 and OAS1 (2',5'-oligoadenylate synthetase) and VIM (vimentin). The highest degree gene was TP53, with the degree 146. In addition, a total of 1057 nodes and 4729 edges (data not shown) were analyzed by MCODE, a plug-in unit in cytoscape. Top 5 significant modules were selected and corresponding functional and pathway enrichment analysis were performed (Figure 3). The pathways that genes in module 1–5 associated with are displayed in Table 3. In survival analysis, high mRNA expression of SRC was linked

Table 2. The top 15 DEGs with higher scores identified by the three centrality methods respectively.

Gene	Degree	Gene	Subgraph	Gene	Closeness
TP53	146	ISG15	6.7E+10	TP53	0.054726
SRC	119	STAT1	5.3E+10	SRC	0.054704
IL6	89	OAS1	5.09E+10	JUN	0.054235
JUN	75	OAS3	5.09E+10	IL6	0.054146
ISG15	68	HERC5	4.4E+10	VIM	0.053798
CAD	68	GBP1	3.89E+10	CAD	0.053708
STAT1	55	IFIT1	3.88E+10	ISG15	0.053699
OAS3	55	IFIT3	3.8E+10	NOS3	0.053699
OAS1	55	IFIH1	3.8E+10	CDKN2A	0.053634
VIM	52	XAF1	3.52E+10	EDN1	0.053604
ACTR1A	48	RSAD2	3.43E+10	STAT1	0.053593
HERC5	48	CXCL10	3.42E+10	FN1	0.05355
DNAJC10	45	IFI44	3.13E+10	RB1	0.053544
SH3GL1	45	DDX60	3.09E+10	EEF2	0.053514
EDN1	45	TP53	3.06E+10	PDGFRA	0.053514

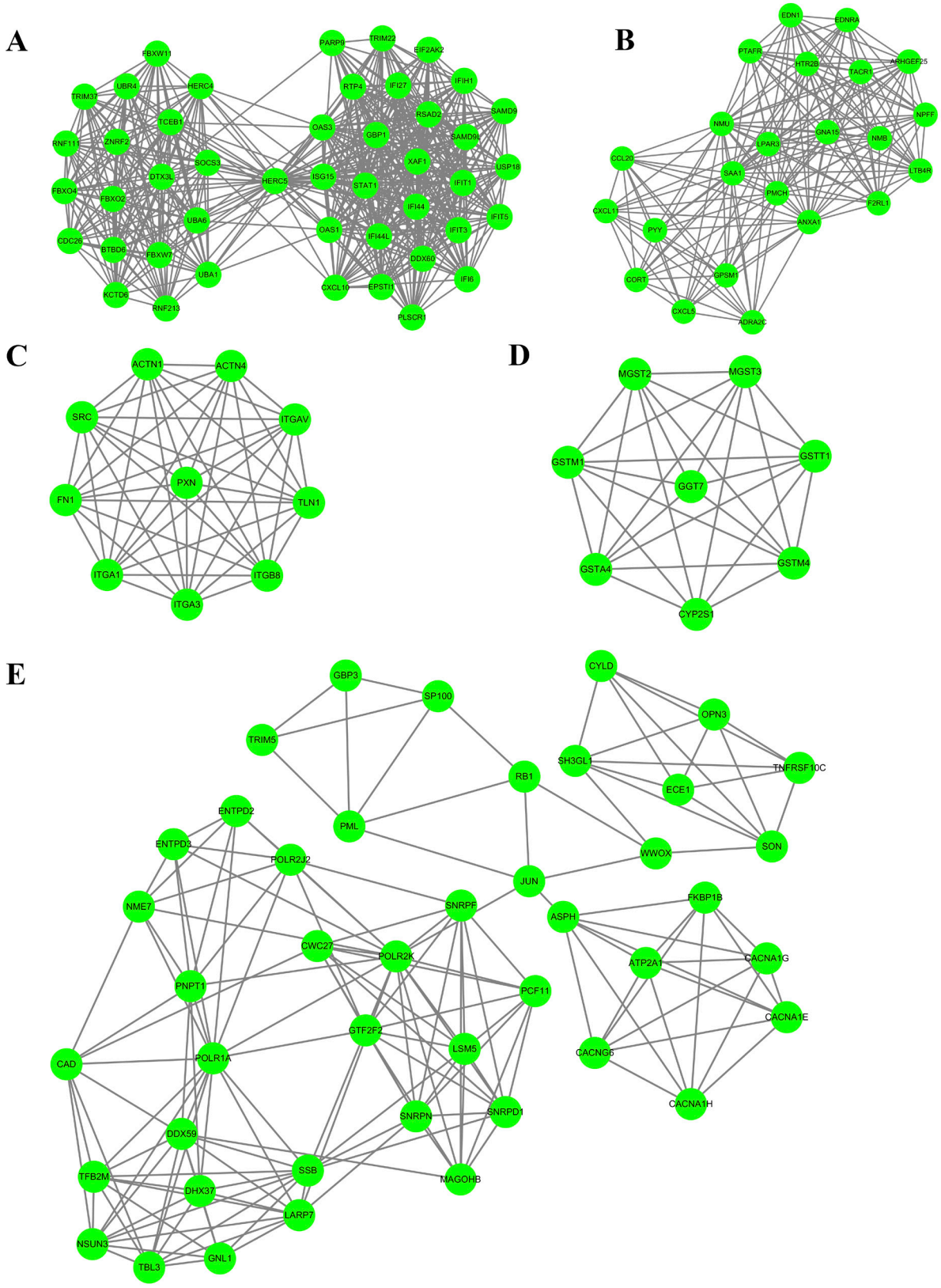


Figure 3. Top 5 modules from the protein-to-protein interaction network. A) module 1, B) module 2, C) module 3, D) module 4, E) module 5, (a) the notation for module 1 (b) the notation for module 2, (c) the notation for module 3, (d) the notation for module 4, (e) the notation for module 5.

with worse OS for PDAC patients, as well as IL-6, ISG15, STAT1, OAS3, OAS1 and VIM (Figure 4).

Discussion

Progression of PDAC is promoted by desmoplasia that is mostly induced by PSCs and the interaction between PDAC cells and PSCs is receiving increasing attention [14, 30]. Understanding the molecular mechanism of interaction between PCCs and PSCs is of critical importance for diagnosis and treatment of PDAC. Since microarray and high-throughput sequencing can provide expression levels of thousands of genes in human genome simultaneously, it has been widely used to predict the potential therapeutic targets for PDAC [31]. In the present study, we identified 1881 upregulated and 1776 downregulated DEGs between BXPC-3 co-cultured with or without primary PSCs using bioinformatic analysis. Function annotation showed that these DEGs were mainly involved in peptide metabolic process, response to stress, signaling and multicellular organismal development.

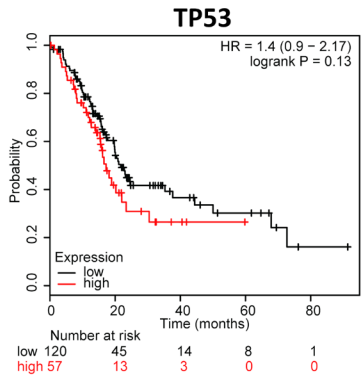
Tumor cells have their own unique metabolic pattern and peptide metabolic process is a vital component. Recent study argued that amino acid glutamine metabolic process played an important role in PDAC and its metabolic pathway may be a novel therapeutic target [32]. PDAC is usually associated with high levels of stress including psychological stress and it was demonstrated to be associated with increased mortality. The relative genes and pathways have been preliminarily identified and deserve further study in the future [33]. As for signaling, it is known that the interaction between PSCs and PDAC cell is based on a complicated signaling pathway network and monitoring these pathways could help prediction of tumor progression.

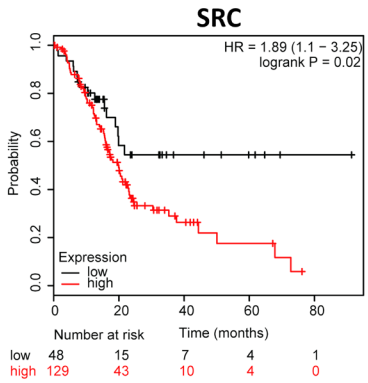
By constructing the PPI, we identified several key genes that can provide new ideas for the therapeutic studies in PDAC: TP53, SRC, IL6, JUN, ISG15, CAD, STAT1, OAS3, OAS1 and VIM. Three centrality methods including degree, closeness and subgraph centrality were applied for research (Table 3).

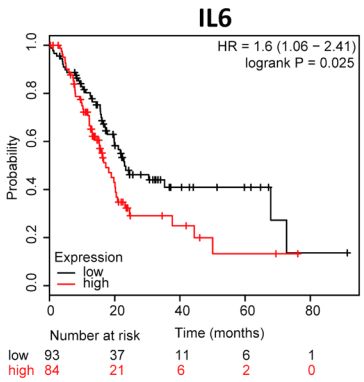
TP53 is one of the most important tumor suppressor genes which is mutated in over 50% of human malignancies [34]. Mutations of TP53 occur in up to 70% of pancreatic adenocarcinomas. The lack of functional TP53 has been proposed to be a component of resistance to DNA-damaging agents, resulting in the inhibition of apoptosis [35]; patients

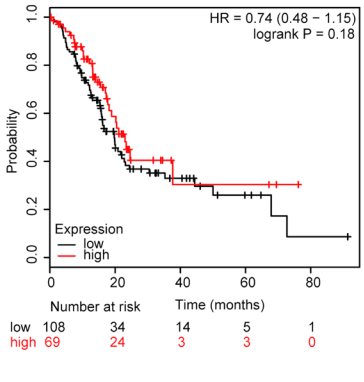
Table 3. The annotation for module 1–5.

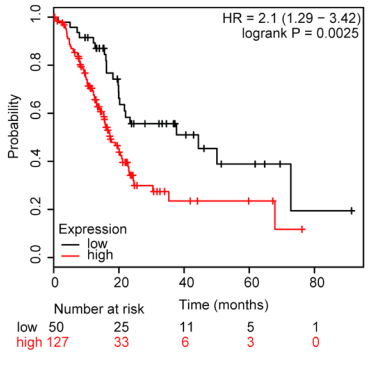
Term	FDR	p-value	Genes
Module 1			
Antiviral defense	1.43E-23	1.62E-20	IFIH1, OAS3, HERC5, RSAD2, IFI44L, OAS1, STAT1, TRIM22, IFIT3, PLSCR1, IFIT1, ISG15, DDX60, IFIT5, EIF2AK2, GBP1
Ubl conjugation pathway	3.54E-17	4.00E-14	SOCS3, DTX3L, FBXO2, UBR4, HERC5, UBA6, HERC4, CDC26, TRIM22, RNF213, ZNRF2, TRIM37, FBXW7, USP18, ISG15, UBA1, FBXO4, TCEB1, FBXW11, RNF111
type I interferon signaling pathway	3.82E-14	5.08E-11	IFIT3, IFI27, IFIT1, ISG15, OAS3, RSAD2, OAS1, XAF1, STAT1, IFI6
Module 2			
Amidation	1.46E-04	1.42E-07	PMCH, NMB, PYY, NMU, NPFF
neuropeptide signaling pathway	2.36E-04	1.73E-07	LTB4R, PMCH, NMB, PYY, NMU, NPFF
Neuroactive ligand-receptor interaction	1.77E-04	2.15E-07	EDNRA, LTB4R, TACR1, F2RL1, LPAR3, ADRA2C, HTR2B, PTAFR
Module 3			
Focal adhesion	1.43E-11	1.57E-14	TLN1, ACTN4, ITGB8, ITGAV, ITGA1, ACTN1, ITGA3, SRC, PXN, FN1
integrin binding	4.27E-09	4.15E-12	TLN1, ACTN4, ACTN1, ITGA3, SRC, PXN, FN1
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	1.11E-05	1.21E-08	ACTN4, ITGB8, ITGAV, ITGA1, ACTN1, ITGA3
Module 4			
glutathione derivative biosynthetic process	3.59E-11	4.96E-14	GSTM1, MGST3, GSTA4, GSTM4, GSTT1, MGST2
Glutathione metabolism	4.55E-10	8.31E-13	GSTM1, MGST3, GGT7, GSTA4, GSTM4, GSTT1, MGST2
Metabolism of xenobiotics by cytochrome P450	4.67E-09	8.52E-12	GSTM1, MGST3, GSTA4, GSTM4, CYP2S1, GSTT1, MGST2
Module 5			
mRNA splicing, via spliceosome	1.94E-03	1.38E-06	PCF11, POLR2K, SNRPN, CWC27, GTF2F2, SNRPD1, LSM5, SNRPF
Pyrimidine metabolism	2.44E-03	2.30E-06	POLR2K, PNPT1, POLR1A, CAD, ENTPD3, POLR2J2, NME7
RNA binding	9.04E-03	7.77E-06	SON, SNRPN, LARP7, DDX59, PNPT1, SNRPD1, LSM5, SSB, NSUN3, SNRPF

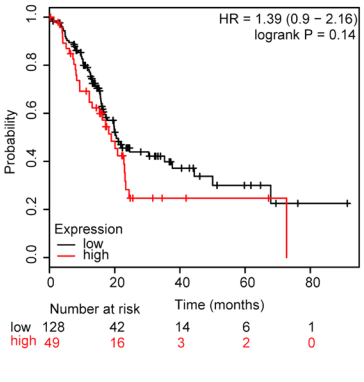

TP53
HR = 1.4 (0.9 − 2.17)
logrank P = 0.13
Probability
Expression
low
high
Time (months)
Number at risk
low 120 45 14 8 1
high 57 13 3 0 0

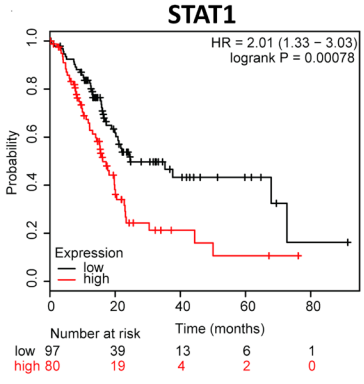

SRC
HR = 1.89 (1.1 − 3.25)
logrank P = 0.02
Probability
Expression
low
high
Time (months)
Number at risk
low 48 15 7 4 1
high 129 43 10 4 0

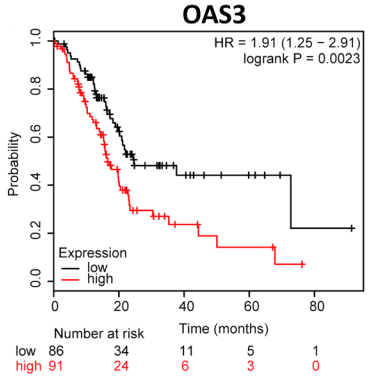

IL6
HR = 1.6 (1.06 − 2.41)
logrank P = 0.025
Probability
Expression
low
high
Time (months)
Number at risk
low 93 37 11 6 1
high 84 21 6 2 0

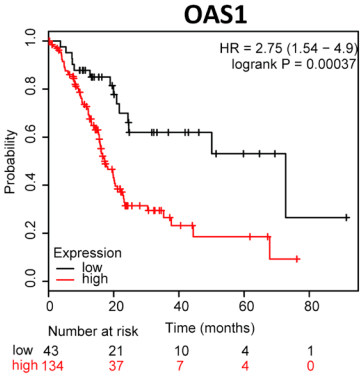

JUN
HR = 0.74 (0.48 − 1.15)
logrank P = 0.18
Probability
Expression
low
high
Time (months)
Number at risk
low 108 34 14 5 1
high 69 24 3 3 0

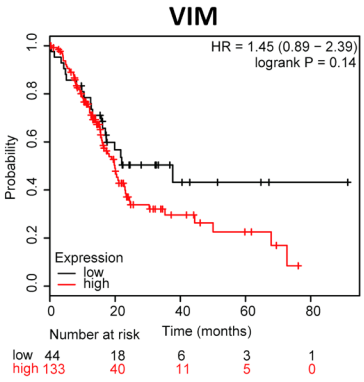

ISG15
HR = 2.1 (1.29 − 3.42)
logrank P = 0.0025
Probability
Expression
low
high
Time (months)
Number at risk
low 50 25 11 5 1
high 127 33 6 3 0


CAD
HR = 1.39 (0.9 − 2.16)
logrank P = 0.14
Probability
Expression
low
high
Time (months)
Number at risk
low 128 42 14 6 1
high 49 16 3 2 0


STAT1
HR = 2.01 (1.33 − 3.03)
logrank P = 0.00078
Probability
Expression
low
high
Time (months)
Number at risk
low 97 39 13 6 1
high 80 19 4 2 0


OAS3
HR = 1.91 (1.25 − 2.91)
logrank P = 0.0023
Probability
Expression
low
high
Time (months)
Number at risk
low 86 34 11 5 1
high 91 24 6 3 0


OAS1
HR = 2.75 (1.54 − 4.9)
logrank P = 0.00037
Probability
Expression
low
high
Time (months)
Number at risk
low 43 21 10 4 1
high 134 37 7 4 0


VIM
HR = 1.45 (0.89 − 2.39)
logrank P = 0.14
Probability
Expression
low
high
Time (months)
Number at risk
low 44 18 6 3 1
high 133 40 11 5 0

with high TP53 expression had a poor overall survival [36]. Saison-Ridinger et al. found that TP53 acted as a regulator of PSC activation and the fibrosis of PDAC. In addition, activated stromal TP53 could reprogram activated PSC to quiescence according to the research [37]. As for the second gene SRC, it is reported that its family proteins in circulating mononuclear cells were viewed as novel biomarkers for PDAC [38]. High expression of SRC has been shown to contribute to several malignant behaviors such as inhibition of apoptosis, enhancement of proliferation and decreased cell-cell, cell-matrix adhesions in PDAC, accompanied with reduced overall survival. Je et al. declared that the knockdown of SRC could reduce the proliferation, migration and invasion of PDAC cells, and SRC kinase (SFK) should be potential target for treatment of PDAC [39]. Dasatinib, a small-molecule SFK inhibitor, has showed a great value in the PDAC treatment [40]. The third key gene IL-6, which encodes a cytokine that functions in inflammation and the maturation of B cells, has been demonstrated to be linked with the interaction between PSCs and PDAC cells. Its related biological functions are based on the inflammatory response, IL-6/JAK/Stat3/NRF2 signaling pathway, epithelial-mesenchymal transition process and autophagy [41, 42]. JUN gene encodes a protein highly similar to the viral protein which was demonstrated to be involved in human malignant disease such as hepatocellular carcinoma, acute myeloid leukemia, colorectal cancer, breast cancer, B-cell lymphomas [43–48]. However, few reports associated with pancreatic cancer cells or PSCs which might be a novel research direction for PDAC. ISG15 is an interferon-inducible ubiquitin-like protein and its expression is highly induced upon viral or bacterial infection. It was demonstrated to have a strong correlation with bladder cancer [49] and to be a possible novel breast tumor marker with prognostic significance [50]. Recently, one study showed that ISG15 could play a role in cancer cell growth inhibition and apoptotic cell death, which provides us with a novel target in treatment of cancer, including PDAC [51]. CAD gene encodes enzymes required for the first three steps in *de novo* pyrimidine synthesis which is necessary for proliferation of tumor [52]. In the development of breast cancer, it was demonstrated to be regulated by ERα/Sp1-mediated pathway [53]. And it was viewed as an early marker of the recurrence of prostate tumor [54]. The main biological function of STAT1 includes immunomodulation [55], cytostatic function [56], cell apoptosis [57], cellular differentiation [58] and suppression of tumor formation [59]. STAT1 is mainly involved in the intervention of antifibrotic interferon-γ (IFNγ) effects in PSCs which plays a vital role in chronic pancreatitis and pancreatic cancer [60]. One research showed that IFNγ-induced STAT1 signaling pathway could hit both PSCs and cancer cells [61] which provided a novel ideal for the treatment of pancreatic cancer. And a recent study uncovered that the inhibition of Jak/STAT signaling could reduce PSC activation and decrease the severity of chronic pancreatitis [62]. In addition, this famous signaling pathway has cross-talks with other pathways such as MAPK and NF-κB [63, 64] which is worth our attention. The OAS system is an IFN-induced antiviral pathway and OAS gene is known as interferon stimulated genes (ISGs) [65, 66]. There are all three OAS proteins in humans: OAS1, OAS2 and OAS3. In current research, OAS1 and OAS3 showed higher degrees in PPI network. Oncolytic virus (OV) therapy is a promising strategy to deal with pancreatic cancer, however, cell lines displaying high-level expression of OAS showed resistance to this therapy [65]. Therefore, OAS could be a novel biomarker to identify suitable pancreatic cancer patient. What's more, OAS could be a potential immunotherapy target in pancreatic disease, such as type 1 diabetes [67] and pancreatic cancer [68]. In addition, previous research showed that genetic variation in OAS3 could influence the risk of chronic lymphocytic leukemia [69] and OAS1 was proven to be related to prostate cancer [70]. Vimentin (VIM) is known as a mesenchymal marker that plays a vital role in epithelial mesenchymal transition in malignant tumors including PDAC. Vimentin has been reported to be related to several pathways such as cell adhesion, cytoplasmic microtubule assembly and cytoskeleton remodeling [71]. High expression of vimentin in PDAC was associated with higher metastatic ability and higher degree of malignancy, which means poorer outcome of the patient. In addition, the vimentin methylation status could be a valuable predictor in PDAC, and it needs more clinical trials for further evidence [71, 72].

Module analysis of the PPI network uncovered that the interaction between PSCs and pancreatic cancer cells was mainly associated with "antiviral defense", "type I interferon signaling pathway", "focal adhesion", "glutathione derivative biosynthetic process" et al. Cancer immunotherapy has been a hotspot these years and antiviral defense plays a vital role. Type I IFN-mediated innate immune response was demonstrated to have effect on the potency of the specific anti-tumor vaccine [73]. Key genes OAS, OAS3 and ISG15 were enriched in this regard, which could be potential immunotherapy target for PDAC. Focal adhesion kinase (FAK) signaling has been proved to play a role during tumor progression such as migration, proliferation and chemokine transcription [74]. In PDAC, single-agent immunotherapy has limited effect owing to the immunosuppressive and fibrotic tumor microenvironment, which were demonstrated to be enhanced by elevated FAK activity. Furthermore, the inhibition of FAK showed its edge in the treatment of PDAC and some FAK inhibitors are in clinical testing now [75].

In conclusion, our data provide a comprehensive bioinformatics analysis of DEGs, which may be involved in the interaction between BXPC-3 and PSCs and reflected the key roles of PSCs in tumor microenvironment on the progression. The study provides a set of useful targets for future investigation into the molecular mechanisms and biomarkers. However, further molecular biological experiments are required to confirm the function of the identified DEGs in PDAC cells.

Supplementary information is available in the online version of the paper.

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