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Research Article

Gene Expression Profiling in Long and Short-Term Survivors after Resection for Pancreatic Cancer Reveals New Insights into Prognosis and Treatment

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Keywords

- Pancreatic cancer
- Gene expression
- Overall survival

Abstract

Background: The ability to identify patients that have disease progression soon after surgical resection could guide treatment as well as aid in the development of novel targeted therapies. This study correlates gene expression and overall survival in patients with pancreatic adenocarcinoma.

Methods: Patients with pancreatic adenocarcinoma treated with definitive surgery without neoadjuvant therapy were grouped into short-term (<10 months, n=13) and long-term (>20 months, n=11) survivors. RNA was extracted from snap-frozen tissues, and global gene expression was examined. Pathway analysis was also performed.

Results: The mean overall survival in each group was 7.5 and 32.0 months. We identified 163 genes that were differentially expressed between patients who survived <10 months and >20 months after definitive surgery. Many of the genes identified have known prognostic importance; however, less than half of these genes have been reported to be associated with survival in pancreatic adenocarcinoma. Pathway analysis identified expression targets of SP1, JUN, and EGF to be highly regulated based upon differences in overall survival.

Conclusion: In pancreatic adenocarcinoma patients who have undergone definitive resection, we have identified multiple genes associated with inferior survival. Many of the genes reported in this study have not previously been linked to overall survival in this patient population.

ABBREVIATIONS

IPMN: Intraductal Papillary Mucinous Neoplasm; OS: Overall Survival; PDAC: Pancreatic Ductal Adenocarcinoma

INTRODUCTION

Pancreatic cancer is a challenging disease with dismal prognosis for the vast majority of afflicted patients. The incidence of pancreatic cancer in the United States is estimated to be 48,960 in 2015 with 40,560 deaths and a 5-year survival rate of 7% [1]. Pancreatic ductal adenocarcinoma (PDAC) accounts for about 90% of the malignant cancers arising from the ductal epithelium in the exocrine part of the pancreas gland. Due to the silent nature of the disease, most patients present late with unresectable disease but approximately 20% will undergo resection followed by chemotherapy and radiation treatment. Disappointingly, despite the use of adjuvant therapy, a significant number of these patients will recur early after resection and die of the disease within one year [2], whereas 25% of these patients with resected PDAC can live for 5 years or more [3]. Traditional prognostic factors including stage, tumor grade, negative surgical margins, and absence of lymph nodes cannot always accurately predict long-term survival. The biology of the tumor may be more important in predicting distant recurrence and ultimately survival. Identifying prognostic factors that can predict which patients may live longer would help with treatment decisions postoperatively but also including the use of neoadjuvant therapy as a means of delaying surgery in patients who would otherwise have rapid disease progression.

One way to select these individuals would be to define a prognostic signature that can identify patients with more aggressive tumor biology prior to treatment. Many different aspects of PDAC tumor biology have been suggested as candidates for determining the aggressive phenotype including genetic [4], epigenetic [5], tumor microenvironment [6], immune response [7] or presence of cancer stem cells (CSCs) [8]. A recent report failed to find a difference in the somatic mutation profile of PDACs

in very long-term survivors compared to PDACs in patients unselected for survival [9]. In the absence of a specific genetic mutation profile that discriminates long-term survivors, another approach is to study gene expression. Expression profiling of PDAC has been undertaken by several different investigators [10,11] and uncovered various signaling pathways associated with tumor progression and metastatic disease of particular interest is the study of Stratford et al. [12], who discovered a six-gene signature that was predictive of survival in localized PDAC in comparison to metastatic PDAC. Interestingly, most genes in the classifier (*SIGLEC11, KLF6, NFKBIZ, ATP4A, GSG1,* and *FOSB*) did not have an obvious role in carcinogenesis, and only three had significantly higher expression in the poor prognostic patients.

Instead of comparing primary PDAC tumors at the extremes of disease (localized versus metastatic), we specifically selected a subgroup of patients who were all considered candidates for surgical resection, and from this cohort we further selected patients with short-term (<10 months) and those with long-term survival >20 months). Our focus was not only to identify genes of interest so as to assist in the development of a specific prognostic gene signature that could guide treatment decisions at presentation and postoperatively but also to identify the key pathways involved in patients with poor outcomes as potential targets for novel treatment strategies.

MATERIALS AND METHODS

Patient consent and sample acquisition

Between February 2009 and November 2013, patients who underwent a pancreatic oduodenectomy for pancreatic adenocarcinoma were approached to submit portions of their tumor to the Beaumont BioBank. A single surgeon completed all resections. In addition to surgery, patients received adjuvant therapy as previously reported [13]. Patients were consented by Beaumont BioBank clinical staff using an IRB approved protocol (HIC 2008-180), and samples were processed and stored at -80°C using standard operating procedures. Analysis was limited to patients who did not present with distant metastasis and did not receive preoperative chemo- or radiation therapy. We identified 11 patients who lived greater than 20 months following their surgery and 13 patients who lived less than 10 months. Inclusion criteria required survival of greater than 100 days following surgery in order to eliminate death due to surgical complications or other comorbidities.

RNA isolation

Frozen pancreatic adenocarcinoma tissue specimens stored at -80°C in RNA later Stabilization Solution (Life Technologies, Carlsbad, CA) were homogenized into lysis buffer using the gentle MACS dissociator's (Miltenyi Biotec Inc., Auburn, CA) "Homogenization of tissue for total RNA isolation" protocol. Following the manufacturer's protocol, RNA was purified using the E.Z.N.A. Total RNA Kit I (Omega, Norcross, GA), quantified (Nanodrop 8000, Thermo Scientific), and then stored at -80°C. RNA integrity was determined by Bioanalyzer analysis (Agilent) just prior to processing for expression microarray analysis.

Illumina expression beadchips

RNA was amplified and labeled using the TargetAmp-Nano Labeling Kit (Epicenter, Madison, WI) which enables amplification and target preparation compatible with the Direct Hybridization Assay (Illumina, San Diego, CA). Amplification was performed with 500ng of total RNA input following procedures described in the TargetAmp-Nano Labeling Kit user guide. Hybridization and staining to the HumanHT-12 v4 Expression BeadChip (Illumina, San Diego, CA) was performed using 750ng of Biotin-aRNA product following protocols outlined in the Whole-Genome Gene Expression Direct Hybridization Assay Guide. Subsequent scanning of the BeadChip was performed using the iScan Microarray Scanner (Illumina, San Diego, CA).

Gene expression and pathway analysis

Gene expression data from 24 samples were imported into Illumina's Genome Studio. They were imported with cubic spline normalization. Quality control was performed in Genome Studio. The Partek Report Plug-in from Illumina's Genome Studio was used to export the gene expression data from 26 arrays into Partek's Genomics Suite (version 6.15.1207). Differentially expressed genes were detected by ANOVA ($p \leq 0.01$ and 2-fold cutoff) taking into account the parameters of survival and barcode. Barcode refers to the chip used; it is included to account for hybridization differences associated with runs on different bead chips. Pathway analysis was done with Pathway Studio (Elsevier, version 11.1.0.6 2015-12-08). The data are available using NCBI Gene Expression Omnibus accession number GSE77435 [14].

RESULTS

Patient clinical data

Table (1) lists patient characteristics, pathologic findings, additional treatments, and overall survival (OS) from the time of surgery. The median age was 64 years, and 50% of patients were male. The mean OS after surgery for all patients in the study was 18.7 months (median: 9.5 months) with a mean of 32.0 months (median: 25.6 months) and 7.5 months (median: 8.5 months) for the >20 months and <10 months groups, respectively. There was no significant difference in the pathologic T (T3=77% vs. 82%; p=0.77) or N (N1=85% vs. 82%; p=0.85) stage or positive surgical margins (31% vs. 9%; p=0.19) between patients based upon OS. The <10 month survival group had a significantly larger percentage of poorly differentiated tumors (77% vs. 36%; p=0.04). There was also a difference in postoperative (p=0.04) treatment.

Gene expression differences at >20 months

We identified 163 genes that were differentially expressed ($p \leq 0.01$ and 2-fold cutoff) between patients who survived <10 months and patients with survival >20 months (Figure 1) (Table 1). This included genes associated with epithelial to mesenchymal transition, vascularization, and cell migration (Table 2). Some of the greatest increases in expression for short-term survivors were seen in *KRT17, S100P, LCN2, COL17A1, and COL1A1* amongst others whilst some of the most prominent genes that were down regulated in the long-term survivors included *GSTA1*, *GSTA2*, *LGALS2*, and *CXCL9*.

Signaling changes at >20 months

Pathway Studio utilizes a literature mining tool, MedScan, to

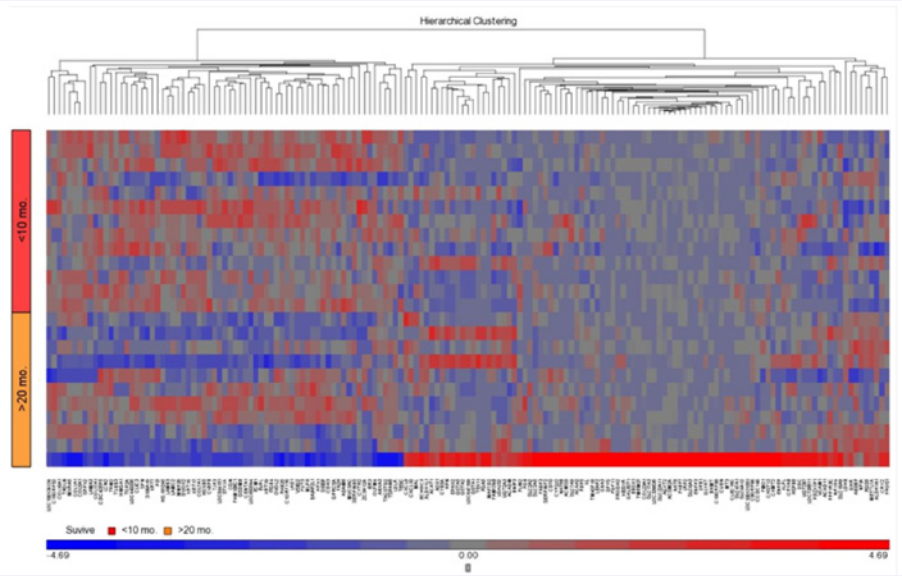

Figure 1 Heatmap illustrating the 163 genes that are differentially expressed (p≤0.01 and 2-fold) between patients who survived <10 months and those that survived >20 months.

Table 1: Patient Characteristics.

Age at resection (years)	**Patients with OS <10 months OS <10 months from resection (n=13)**	**Patients with OS >20 months from resection (n=11)**	***P***
Median	63.6	64.3	0.89
Range	41-79	51-82	
Gender, n (%)			
Male	7 (54)	5 (45)	0.68
Female	6 (46)	6 (55)	
Location in pancreas, n (%)			
Head only	9 (69)	10 (91)	0.53
Body only			
Tail only	2 (15)		
Body and Tail	1 (8)	1 (9)	0.04
Head, body, and tail	1 (8)		
Grade, n (%)			
Poorly differentiated	10 (77)	4 (36)	0.77
Moderately differentiated	3 (23)	7 (64)	
pT stage, n (%)			
pT2	3 (23)	2 (18)	
pT3	10 (77)	9 (82)	0.85
pN stage, n (%)			
pN0	2 (15)	2 (18)	
pN1	11 (85)	9 (82)	0.19
pM stage, n (%)			
pM1	0 (0)	0 (0)	
Surgical margin, n (%)			
R0	9 (69)	10 (91)	
R1	4 (31)	1 (9)	0.04
None	13 (100)	11 (100)	
Type of postoperative treatment, n (%)			
Chemotherapy	2 (15)	3 (27)	
Chemoradiotherapy	5 (38)	8 (73)	<0.0001
None	1 (8)		
Unknown	5 (38)		
Mean OS from resection, mo. (standard deviation)			
	7.5 (1.6)	32.0 (9.6)	

generate sub-networks that associate genes with other entities such as cell processes. A Fisher's Exact test was used to identify sub-networks that are highly represented by differentially expressed genes. One type of sub-network associates genes with a central seed based upon the ability of the seed to control gene expression. The top expression target sub-networks that were highly represented by the differentially expressed genes are listed in Table (3), including the expression targets of SP1, JUN, and PPARG (Figure 2) of note is the general downregulation of PPARG expression targets and the presence in these sub-networks of *COL1A1*, COL7A1, *GPRC5A, KRT17, and ECM1* which have not been previously linked to patient outcomes in PDAC. Table (4) lists the top sub-networks of genes regulating cell processes. Differentially expressed genes between patients with OS <10

Gene expression differences at >20 months

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Signaling changes at >20 months

Pathway Studio utilizes a literature mining tool, MedScan, to generate sub-networks that associate genes with other entities such as cell processes. A Fisher's Exact test was used to identify sub-networks that are highly represented by differentially expressed genes. One type of sub-network associates genes with a central seed based upon the ability of the seed to control gene

Table 2: Selection of genes differentially expressed (p≤0.01 and 2-fold cutoff) in patients who survive <10 months compared to those with survival >20 months. Fold change is short survival compared to long survival. Red parentheses represent downregulation in short survival patients. Complete list of genes found in Table (1).

E-M Transition		**Vascularization**		**Cell Migration**	
COL1A1	4.19	ANXA3	2.20	ALDH1A1	–2.58
COL5A1	3.13	BNIP3	–2.87	BAIAP2L1	2.05
CRYAB	–2.86	CD70	–2.62	CLIC3	2.68
CXCL10	–2.77	COL17A1	4.77	COL16A1	2.26
CYP2J2	–2.65	COL7A1	3.28	COL23A1	–2.01
ECM1	2.16	CXCL9	–3.06	ENPP3	–2.41
EPHX2	–2.06	CYTH2	2.12	FA2H	2.17
HMGA1	2.23	ENPEP	–2.15	FABP3	–2.13
IER3	2.19	FGB	–2.76	FABP7	–3.09
ITGB4	2.15	HOXB5	2.16	FAM134B	–2.10
KISS1R	–2.47	HPN	–2.03	GPRC5A	2.76
KL	–2.10	KRT17	6.90	GSDMB	2.20
KRT19	2.67	LAMB3	2.74	HSPB8	–2.35
LAMC2	2.79	LDLR	2.55	LDHB	–2.06
LCN2	5.74	NR1H4	–2.43	LRP2	–2.32
MMP28	2.45	PROS1	–2.04	MUC5AC	4.26
OVOL2	2.23	SLC2A2	–2.05	OLFM4	4.94
RGS5	–2.31	SLC6A3	–2.52		
SERPINB5	2.47	SRPX2	2.29	**Cell Growth**	
SERPINF2	–2.23	TSPAN8	3.52	ABI3BP	–2.36
TACSTD2	2.85	TUBB3	2.23	ALDH1L1	–2.06
TAGLN	2.59	ZC3H12A	2.02	ALPL	–2.28
VCAM1	–2.12			FMO1	–2.67
		Differentiation		GSTA1	–4.32
Proliferation		ACADM	–2.14	KRT7	3.33
BHMT	–3.29	AOX1	–2.46	MT3	–2.77
CYB5A	–2.91	CENTA1	2.34	NFIA	–2.11
FGF11	–2.05	CGN	2.31	PITX1	3.44
PPP1R3C	–2.05	CUBN	–2.55	PLIN2	–2.81
TJP3	2.37	DDC	–2.53	TEX11	–2.83
		KRT16	2.82	TMEM27	–3.48
		MLPH	3.19	TOP2A	2.10
		SLC2A5	–2.18		
		STX1A	2.16		
		TESC	2.76		

expression. The top expression target sub-networks that were highly represented by the differentially expressed genes are listed in Table (3), including the expression targets of SP1, JUN, and PPARG (Figure 2) of note is the general downregulation of PPARG expression targets and the presence in these sub-networks of *COL1A1*, COL7A1, *GPRC5A, KRT17, and ECM1* which have not been previously linked to patient outcomes in PDAC. Table (4) lists the top sub-networks of genes regulating cell processes. Differentially expressed genes between patients with OS <10 months and those with OS >20 months are highly represented by genes regulating cell differentiation, cell proliferation, cell migration, and vascularization (Supplemental Table 1).

Comparison to survival at >37 months (1100 days)

With the aim of further isolating gene expression that is associated with longer survival, analysis was then focused on the patients that demonstrated the longest survival. When patients with OS <10 months were compared to the subset of the longest surviving patients (>37 months, n=4), there were 196 differentially expressed genes. Of these, 45 genes Table (2), were differentially expressed in both the comparison of <10 months to >20 months and <10 months to >37 months. Thirteen of these commonly differentially expressed genes are listed in Table (5), along with their change in fold expression, purposed function, and known prognostic importance. In addition to these individual gene expression differences, sub-networks of genes associated with regulating cell differentiation, cell proliferation, cell adhesion, and cell migration were highly represented at both time points Table (3). The expression target sub-networks of SP1, JUN, and EGF were highly represented at both time points Table (3) and Figure (1). In Figure (3), the data was presented to illustrate the gene expression differences between the two comparisons. Several upregulated genes (*PI3, SFN, CLIC3, KRT16, S100A16, KRT19, EVPL, ECM1, DUOX2*) demonstrated continued escalation of expression with increased OS. Of the downregulated genes, *LGALS, CXCL9, AOX1, TEX11, ALDH1A1, NFIA, METTL7A, and FAM134B* showed decreasing levels of expression as OS progressed from 20 to 37 months (Supplemental Table 2).

DISCUSSION

This study reports on pancreatic adenocarcinoma gene expression differences in patients who survived greater than 20 months or less than 10 months following surgery. There was no significant difference in the age, sex, or stage between the two groups, and no one received preoperative therapy. There was, however, a significantly greater percentage of high grade tumors in patients who lived <10 months.

Table 3: Expression target sub-networks identified as highly represented by the 163 genes that are differentially expressed in patients that live >20 months versus those that live <10 months. Overlap indicates the number of differentially expressed genes (p≤0.01 and 2-fold) in the sub-network.

Expression Targets of:	Overlap	p-value
SP1	38	3.35E-11
Jun/Fos	29	1.25E-10
JUN	21	1.11E-08
PPARG	23	1.26E-08
cytokine	31	1.65E-08
MAPK1	26	5.83E-08
MAPK8	21	7.20E-08
HNF1A	13	1.40E-07
TNF	36	3.08E-07
EGF	22	5.29E-07

Table 4: Sub-networks of genes regulating cell processes identified as highly represented by the 163 genes that are differentially expressed in patients that live >20 months versus those that live <10 months. Overlap indicates the number of differentially expressed genes (p≤0.01 and 2-fold) in the sub-network.

Gene Set Seed	Overlap	p-value
cell differentiation	74	1.31E-18
cell invasion	48	8.61E-18
cell proliferation	83	3.04E-16
cell migration	55	1.70E-15
cell adhesion	43	2.45E-14
cell behavior	46	1.15E-13
cell motility	34	3.16E-13
Angiogenesis	43	1.31E-11
pregnancy	30	1.34E-11
cell survival	45	1.94E-11

Traditional prognostic criteria for long-term survivors have included negative margin status, small tumor size, no lymph node involvement, low CA 19-9 level, low grade, absence of metastases, and type of treatment administered [15-17]. However, the use of these prognostic factors has limited value due to the heterogeneity of the long-term survival group. Ferrone et al. showed that negative margins and negative nodes demonstrated a positive prognosis, but at the same time 41% of long-term survivors had positive nodes and 24% had positive margins [18]. Adham et al., found that typically positive prognostic criteria did not predict long survival with 29 of 30 long-term survivors having T3/T4 tumors with 12 of 30 having positive lymph nodes [19].

One possible conclusion is that the biology of the tumor, not traditional prognostic markers, is important for prediction of long-term survival. One recent study by Dal Malin et al., tried to address this by using next-generation exome sequencing to examine the genomic profile of long-term survivors [9]. While mutations were found in KRAS, TP53, SMAD4, and CDKN2A, there were no mutations that were preferentially found in the long-term survivors.

In order to continue the search for the biological variability seen in the long-term survivors, we have identified 163 genes that were differentially expressed between the <10 months and >20 months survival groups. Several of the genes we identified have a known prognostic role in pancreatic adenocarcinoma including ADAM metallopeptidase domain 8 (*ADAM8*) and transgelin (*TAGLN*) along with aldehyde dehydrongenase 1 family, member A1 (*ALDH1A1*). However, most of the genes we identified have not been previously linked to prognosis in pancreatic adenocarcinoma.

Aldehyde dehydrogenase 1 family, member A1 is an enzyme involved in alcohol metabolism. It was found to have decreased expression (2.6-fold) in short-term survivors compared to the

Table 5: Characteristics of genes differentially expressed in patients demonstrating longer survival after definitive surgery. Fold change is short survival (<10 months) compared to long survival (>20 months). Red parentheses represent downregulation in short survival patients.

Gene Symbol Enzyme	Fold change	Name	Function	Importance in PDAC	Role in other malignancies
ADAM8	2.8	ADAM 2.8 metallopeptidase domain 8	Implicated in a variety of biological processes involving cell-cell and cell-matrix interactions	Elevated expression is associated with reduced survival time in PDAC patients [46]. Also implicated in increased invasiveness in cell culture [47].	Overexpression correlates with poor survival in patients with hepatocellular carcinoma, medulloblastoma, osteosarcoma, and glioma [48-51].
ALDH1A1	–2.58	Aldehyde dehydrogenase 1 family, member A1	Enzyme in the major pathway of alcohol metabolism	Low expression is a poor prognostic marker in PDAC [24]. Implicated in gemcitabine resistance and tumorigenesis in vitro [25, 26].	Marker of poor prognosis in urothelial bladder, colon, breast, NSCLC, esophageal, oral squamous cell carcinoma, papillary thyroid, and vulvar squamous cell carcinoma [20-23,52-55].
GSTA1	–4.32	Glutathione S- transferase α1	Function in the detoxification of electrophilic compounds	None reported	Linked to survival in breast cancer [56], recurrence risk after chemotherapy for Hodgkin lymphoma [57], and susceptibility to hepatocellular carcinoma and urothelial carcinoma [58,59].
Receptor					
GPRC5A	2.76	G protein-coupled 2.76 receptor, class C, group 5, member A	A member of the type 3 G protein-coupling receptor family, characterized by the signature 7-transmembrane domain motif	None reported	Overexpression is associated with shorter survival in hepatocellular carcinoma patients [60].
Cell adhesion and structure					
KRT17	6.9	Keratin 17	Expressed in nail bed, hair follicle, sebaceous glands, and other epidermal appendages	None reported	High expression levels are associated with worse survival in cervical squamous cell carcinoma, epithelial ovarian cancer, breast cancer, and gastric cancer [27-30].
COL1A1	4.19	Collagen, type I, α1	The pro-alpha1 chains of type I collagen	None reported	Linked to poor survival in hepatocellular carcinoma [61].
COL7A1	3.28	Collagen, type VII, α1	Composed of three identical alpha collagen chains, and is restricted to the basement zone beneath stratified squamous epithelia	None reported	Decreased expression correlates with improved survival in esophageal squamous cell carcinom [62].
KRT16	2.82	Keratin 16	A member of the keratin gene family	None reported	Expression is associated with chemoresistance in triple negative breast cancer [63].
LAMB3	2.74	Laminin β3	Belongs to a family of basement membrane proteins	None reported	Decreased expression correlates with improved survival in esophageal squamous cell carcinoma [62].
TAGLN	2.59	Transgelin	A transformation and shape-change sensitive actin cross-linking/ gelling protein found in fibroblasts and smooth muscle	High expression levels are linked to shorter survival in pancreatic adenocarcinoma [64].	Marker of metastatic potential and shorter overall survival in colorectal cancer [65 66].
ECM1	2.16	Extracellular matrix protein 1	Soluble protein involved in endochondral bone formation, angiogenesis, and tumor biology	None reported	Overexpression is associated with worse survival in hepatocellular carcinoma, disease specific survival in breast carcinoma, and increased metastatic potential of laryngeal carcinoma [67-69].
Unknown function					
SRPX2	2.29	Sushi-repeat containing protein, X-linked 2	None listed	None reported	Increased expression correlates with worse overall survival in gastric cancer [70].
LGALS2	–3.92	Lectin, galactoside-binding, soluble, 2	Soluble beta-galactoside binding lectin	None reported	Decreased expression is associated with lymph node metastasis in gastric [71].

longer >20 months OS patients. This gene has been previously linked to prognosis and progression in many cancers [20-23]. Expression of this gene has also been linked to cancer stem cells (CSCs), with low expression associated with gemcitabine resistance and poor prognosis in pancreatic adenocarcinoma [24-26]. Keratin 17 is an intermediate type I filament chain keratin usually expressed in the nail bed, hair follicle, and sebaceous glands. This gene was found to have higher expression (6.9 fold) in the short-term OS patients. Increased expression of this gene has been linked to poor survival in cervical squamous cell carcinoma, epithelial ovarian cancer, gastric cancer, and breast cancer [27-30]. However, this has not been previously demonstrated to be prognostic in pancreatic cancer.

This study adds to the growing literature on the prognostic utility of gene expression patterns for PDAC. The University of Virginia recently published a 13-gene signature that predicts significantly higher risks of mortality in pancreatic adenocarcinoma patients [31]. Of the 13 genes these authors identified, 4 including *TGFA, ELAVL1,* and *MDM2* had been previously shown to be important in pancreatic adenocarcinoma, 6 genes were associated with prognosis or highly expressed in other forms of cancer but not previously reported in PDAC, and 3 had not

been reported to be prognostically significant in any malignancy. Those categorized as low risk by this gene signature had a median overall survival of 14 months compared to 6 months for high risk patients. There was no overlap in the genes identified for their gene signature and the genes identified in the current study. A 6-gene prognostic signature was also published by the University of North Carolina [12]. This signature included FOSB, NFKBIZ, IKBZ, MAIL, GSGI, and SIGLEC11. Patients classified as low risk by this study had a median overall survival of 49 months, and those classified as high risk had a median overall survival of 15 months. Additionally the current results were compared to a pair of studies in NCBI's Gene Expression Omnibus. The original purpose of these published studies was not to examine survival specifically, but both studies included survival data for at least some of the publicly available data. In a study by Van den Broeck et al. [32], microarrays (GEO accession GSE42952) were used to compare patients with good (DFS > 50 months) and poor (DFS < 7 months) outcome. From a study by Yang et al. [33], the data from a subset of patients (GEO accession GSE62452) were used to compare patients with similar outcomes as the current study – patients with OS > 20 months or < 10 months. Of the genes that were identified as differentially expressed in the current study Table (1), 56 were validated through the analysis of the data in these studies. This included genes such as *COL1A1*, *COL5A1*, *COL7A1*, *CYB5A*, and *STX1A* that were confirmed by both studies. Even more striking is the concordance in regulated cell processes between our current study and the publicly available data. Of the highly regulated cell processes that we identified (Table 4), five of the top six were found in both external studies. This included cell differentiation, cell invasion, cell proliferation, cell migration, and cell behavior which were all highly ranked in both publicly available datasets (Supplemental Table 3).

In addition to individual gene expression biomarkers, signaling surrounding SP1, JUN, and EGF was highly altered in the patients that showed longer survival. This result confirmed the role of these signaling pathways in pancreatic cancer. SP1 is a negative prognostic factor that plays a role in cell proliferation and metastasis [34]. In particular, SP1 protein was found to be overexpressed in a subset of primary pancreatic adenocarcinoma that developed lymph node metastasis, were higher stage and grade, and had a much shorter overall [35]. JUN is a transcription

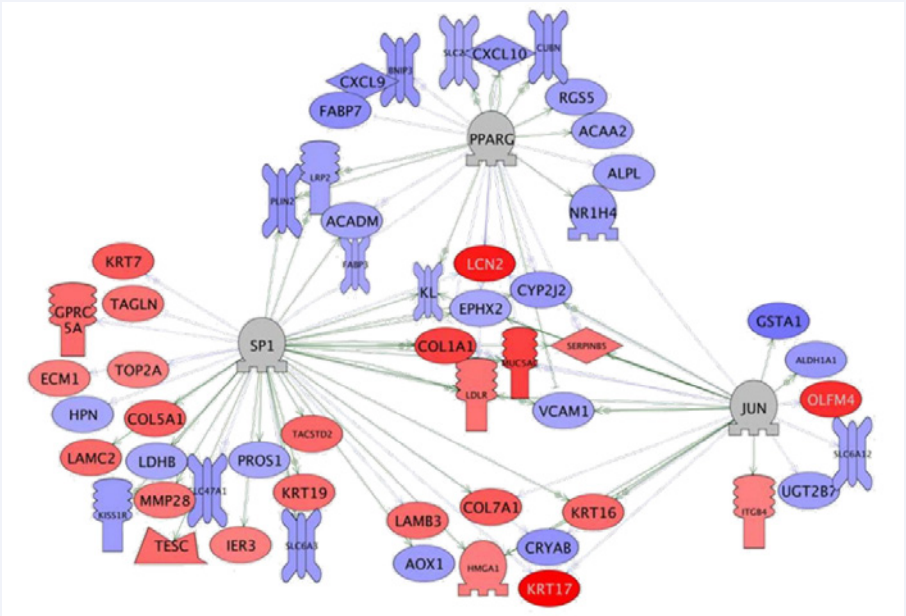

Figure 2 Combined expression target sub-networks of SP1, JUN, and PPARG identified as highly represented in the list of genes differentially expressed (p≤0.01 and 2-fold) between patients who survived <10 months and those that survived >20 months. Genes in red are upregulated in patients with shorter survival; genes in blue, downregulated.

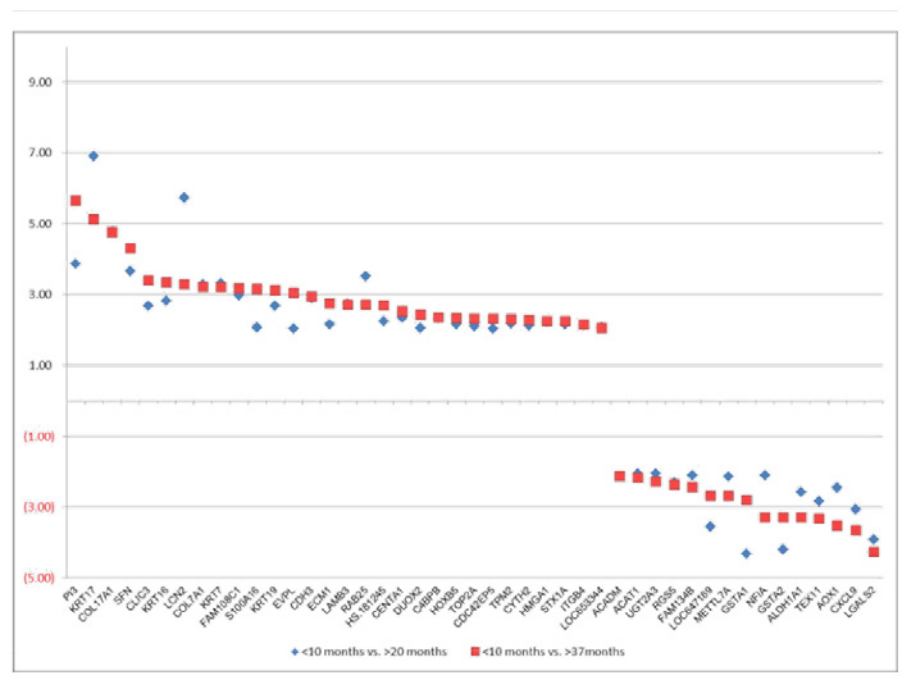

Figure 3 (A) – Gene expression changes in patients surviving greater than 20 and greater than 37 months compared to less than 10 months. Genes were initially identified as differentially expressed (p≤0.01 and 2-fold cutoff) in patients with OS >20 months. Blue diamond: OS <10 months versus OS >20 months. Red square: OS <10 months versus OS >37 months.

factor involved with cell proliferation that has been identified as an oncogene. It has previously been related to pancreatic cancer stage, grading, and invasion [36]. Expression of JUN was shown to be elevated in liver metastases compared to pancreatic cancer tissue, and high expression was seen more often in short-term survivors [37]. EGF acts via its receptor (EGFR) to potentiate growth, proliferation and differentiation of many different cell types. Specifically, it has been shown to be involved in growth, invasiveness, and metastasis of pancreatic cancer [38,39]**(Supplemental Figure 1).**

Also of interest is the altered TGFB1 signaling in the patients that showed the longest survival (>37 months). This confirmed the role of this pathway in pancreatic cancer. Patients with high serum levels of TGFβ1 demonstrated significantly reduced survival time [40]. Likewise, TGFβ1 protein expression in PDAC tumor tissue is correlated with overall survival and has been shown to promote cell growth and invasion [41]. In an earlier study by our group, we found that TGF signaling was de-regulated in high grade and invasive intraductal papillary mucinous neoplasms (IPMN) compared to low and moderate grade IPMN [42]. SMAD has also been linked with PDAC. SMAD4 loss via mutation is typically associated with worse OS and associated with distant metastasis while intact SMAD4 is associated with local recurrence [43,44]. While SMAD4 expression was previously shown to not be prognostic for overall survival [40], its expression in tumor epithelial cells was associated with low T stage and with abundant stroma [45]. Interestingly the same study found that expression of SMAD4 by the fibroblast component of the tumor was associated with decreased overall survival. This may explain how in the current study that TGF/SMAD signaling pathways are deregulated whereas the expression of the individual genes – *TGFB1*, *TGFB2*, *SMAD3*, and *SMAD4* – are unchanged.

CONCLUSION

In this comparison of patients who live <10 months and >20 months following definitive resection for pancreatic adenocarcinoma, we have identified multiple differentially expressed genes. Some of these genes have previously been shown to be prognostic in pancreatic adenocarcinoma, but most had never been linked to survival in these patients. These genes and their expression targets warrant further investigation to determine their value as prognostic markers or targets for molecular therapy.

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