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Research Article

Microarray-based identification of differentially expressed growth- and metastasis-associated genes in pancreatic cancer

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Abstract. Pancreatic ductal adenocarcinoma (PDAC) has an extremely poor prognosis. To improve diagnosis and treatment, key mechanisms of deregulated molecular functions have to be identified. Using microarray analysis, the expression patterns of 5600 human genes were assessed in PDAC by comparison with the normal pancreas and chronic pancreatitis (CP). The expression of 467 of 5600 genes was increased in PDAC in comparison to the normal pancreas, and the expression of 120 of these genes was not increased in CP. In addition, 341 of 5600 genes were expressed at decreased levels in PDAC tissues, of which 96 were decreased in comparison to both normal and CP tissues. Thus, a total of 808 of 5600 human genes were differentially expressed in pancreatic cancer. The identification of a large panel of altered genes in PDAC will stimulate additional studies that will lead to improved understanding of the molecular mechanisms underlying pancreatic malignant growth.

Key words. Pancreatic cancer; chronic pancreatitis; DNA array; gene transcription; TGF-β.

Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease with an extremely poor prognosis [1]. Its overall 5-year survival rate is less than 1%, and the median survival time after diagnosis is approximately 5–6 months [2–4]. Clinically, PDAC is often diagnosed at a late stage, when the tumor has already metastasized or invaded surrounding structures, precluding curative resection. Furthermore, it is characterized by unresponsiveness or a low frequency of responsiveness to most conventional treatment options [5, 6]. Even after a potentially curative resection, pancreatic cancers often display rapid local tumor recurrence [7]. Molecular research has contributed significantly to a better understanding of the aggressive nature of this disease, and multiple genetic and epigenetic alterations have been identified in pancreatic cancer over the past decade. According to Hanahan and Weinberg [8] these alterations can be divided into six categories. (i) *Self-sufficiency in growth signals*: PDAC overexpresses a number of mitogenic growth factors and their respective receptors, including members of the epidermal growth factor (EGF) and fibroblast growth factor (FGF) family, which have the potential to stimulate pancreatic cancer cell growth via autocrine and/or paracrine mechanisms [9]. In addition, PDAC also frequently harbors k-ras proto-oncogene mutations [10]. (ii) *Insensitiv-*

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ity to anti-growth signals: PDAC overexpresses members of the transforming growth factor-β (TGF-β) superfamily of ligands [11] and receptors [11–13], but pancreatic cancer cells are often resistant to the growth inhibitory effects normally exerted by these ligands [14]. This resistance is mediated via a number of mechanisms, including Smad4 mutations [15, 16], mutations or deregulated expression of certain receptors, such as activin receptor-IB [17], and TGF-β receptors I and II [14, 18, 19], and increased expression of negative signaling molecules, such as Smad6 and Smad7 [20]. Furthermore, PDAC also displays mutations of the cell cycle inhibitor and tumor suppressor genes $p15^{MTS2}$ and $p16^{MTS1}$ [21]. (iii) *Evading apoptosis*: PDAC displays a high rate of p53 tumor suppressor gene mutations [22] and deregulated expression of apoptosis-regulating molecules including members of the bcl-2 family, such as bcl-2, bcl-x_L, bax, and bak [23, 24], and effectors of apoptosis such as caspases [25]. In addition, pancreatic cancer cells are resistant to apoptosis mediated by members of the tumor necrosis factor-α (TNF-α) family, including TNF-α itself [26], FasL [27], and TRAIL [28], through mechanisms which are not completely known. (iv) *Limitless replicative potential*: PDAC displays increased telomerase activity in contrast to benign pancreatic tumors and pancreatitis samples, which underscores that telomerase activity may be a critical step in pancreatic carcinogenesis [29–31]. (v) *Sustained angiogenesis*: PDAC frequently overexpresses vascular endothelial growth factor (VEGF) and its receptors [32–34] and other growth factors such as FGF-2 and TGF-βs [9], which have been implicated in angiogenesis. (vi) *Tissue invasion and metastasis*: PDAC overexpresses matrix-degrading proteases such as various matrix metalloproteinases [35, 36], the uPA/uPAR system [37], and heparanase [38]. In addition, PDAC frequently displays aberrant expression of integrins [39], cadherins [40], and other adhesion molecules [41].

Most studies of PDAC have focused on specific genes or groups of genes whose function had been previously determined in other cell systems. However, with the development of microarray technology, together with the almost complete sequencing of the human genome, we can now simultaneously assess the expression of a large number of genes in order to identify previously unrecognized disease-specific genes which might serve as potential diagnostic markers or therapeutic targets. Therefore, in the present study, we analyzed alterations in the gene expression patterns of 5600 human genes in PDAC in comparison to the normal pancreas and chronic pancreatitis (CP), utilizing the powerful microarray technology.

Materials and methods

Patients and tissue sampling

Eight PDAC samples were obtained from three male and five female patients (median age 63 years; range 43–80 years). According to the Union Internationale Contre le Cancer (UICC) classification there were two stage II, five stage III, and one stage IV tumors. Eight CP tissue samples were obtained from two male and six female patients (median age 46 years; range 38–51 years). The selected patients were representative of pancreatic cancer and CP patients at our institution. In addition, normal human tissue samples were obtained through an organ donor program from eight previously healthy individuals (five male donors, three female donors; median age 50 years; range 37–69 years). Freshly removed tissue samples were snap-frozen in liquid nitrogen immediately upon surgical removal in the operation room and maintained at –80 °C until use. All studies were approved by the local ethics committee and written informed consent was obtained from all patients.

Oligonucleotide microarray

The GeneChip HuGeneFL array used in this study was fabricated by Affymetrix (Santa Clara, Calif.). It contains about 7000 sequences representing 5600 full-length human genes. The sequences were selected from three databases: exemplars from UniGene were supplemented with additional genes from GenBank and The Institute for Genomic Research (TIGR; Rockville, Md.).

Transcript/expression profiling

Total RNA was extracted by the guanidine isothiocyanate method from snap-frozen human tissues. Poly(A)$^+$ RNA was isolated using oligo(dT)-cellulose kits from Amersham Pharmacia Biotech (Piscataway, N. J.), and 2.5 µg from each preparation was converted into double-stranded cDNA by reverse transcription (GIBCO-BRL Life Technologies, Grand Island, N. Y.) using the T7-T24 primer [5′-GGC CAG TGA ATT GTA ATA CGA CTC ACT ATA GGG AGG CGG (dT_{24})]. The double-stranded cDNA product was purified by phenol/chloroform/isoamyl extraction using phase lock gels (Eppendorf, Westbury, N. Y.). Double-stranded cDNA was converted into cRNA using the in vitro transcription (IVT) MEGAscript T7 kit from Ambion (Austin, Tex.) and biotinylated nucleotides, as previously described [42]. The IVT product was purified using RNeasy mini columns from Qiagen (Valencia, Calif.), and fragmented as described elsewhere [42]. Hybridization of the fragmented IVT product to oligonucleotide arrays was performed as suggested by the manufacturer (Affymetrix, Santa Clara, Calif.).

Computational analysis
Primary analysis of array data was performed using the Affymetrix GeneChip software. Differential expression was defined as an at least threefold change versus respective controls. The detection specificity of the chip used was approximately 1 in 100,000 copies, the difference detection was twofold changes, and the dynamic range was approximately 500-fold.

Statistical analsysis
Data were analyzed by one-way ANOVA using a categorical variable that separated the samples into normal, CP, and cancerous groups. Four hundred and sixty-five genes with changes greater than threefold in two of the possible comparisons were analyzed. When the overall p value of the ANOVA was less than 0.05, individual comparisons between the three groups were performed.

Cluster analysis
Hierarchical clustering was performed on the 1336 genes shown to be 'present' in the study by the Affymetrix algorithm, to determine if the global patterns of expression could discriminate between the three classes of pancreatic tissue sampled. Sample categories were defined as 'normal,' 'cancer,' and 'CP' and represented normal pancreas, cancerous pancreas, and CP samples. The coloring of the figure was not based on a Cy3 : Cy5 ratio, as is typical of these figures, but rather on the rank of the expression level compared to other genes on the chip (with the highest-expressing gene given a value of 1336, the lowest a value of 1). The ranking makes the array results amenable to analysis using Eisen's microarray clustering and display tool [43].

Analysis of the subcellular localization and molecular function of the differentially expressed genes
Three principal resources were utilized. (i) Gene Finder (http://cgap.nci.nih.gov/Genes/GeneFinder) finds genes based on selected search criteria. Each gene is linked to a Gene Info page which provides selected gene data and links to various NCBI and NCI databases. The Gene Finder's Gene Ontology Browser provided the framework for classifying the molecular function and subcellular location of the genes investigated. (ii) The Gene Cards database (http://bioinfo.weizmann.ac.il/cards/index. html) of human genes, their products, and their involvement in disease offers information about the functions of all human genes that have an approved symbol, as well as other selected genes. GeneCards was developed at the Crown Human Genome Center & Weizmann Institute of Science. (iii) The GenBank Database (http://www.ncbi.nlm.nih.gov/) is a DNA sequence database built and maintained by the National Center for Biotechnology Information (NCBI). In addition to the above, PubMed and Medline were also used in the search.

Probe synthesis
For further analysis of the genes of interest, probes were generated by RT-PCR and subsequently subcloned into the pGEMT-easy vector (Promega Biotechnology, Madison, Wis.). Authenticity was confirmed by sequencing. cDNA probes were labeled with α-^{32}P-dCTP using a random-primer labeling system (Roche Diagnostics, Rotkreuz, Switzerland) [11, 20].

Northern blot analysis
Total RNA was extracted by the guanidine isothiocyanate method, size fractionated on 1.2% agarose/1.8 M formaldehyde gels, and stained with ethidium bromide for verification of RNA integrity and loading equivalency, as described previously [11, 20]. RNA was transferred onto nylon membranes and cross-linked by ultraviolet irradiation. Prehybridization, hybridization, and washing were performed under conditions appropriate for cDNA probes, as previously reported in detail [11, 20]. Membranes were exposed at –80 °C to Kodak Bio-Max X-ray films using intensifying screens.

Results

We first analyzed the mRNA levels of 5600 genes in eight human PDAC samples and compared them to eight normal pancreatic and eight CP tissue samples for their absence or presence of expression. This analysis revealed that 56 of 5600 genes were relatively cancer specific inasmuch as their expression was present in at least four of the eight pancreatic cancer samples but only in three or less of the 16 normal pancreatic and CP samples (fig. 1). For 33 (0.6%) of these genes, expression was restricted to pancreatic cancer tissues (table 1). However, none of the genes in this subgroup were expressed in all eight pancreatic cancer samples.
Further quantitative analysis showed that there was an increase in the average expression (defined as an at least threefold change) of 467 genes (8.3%) in pancreatic cancer samples in comparison to normal controls (tables 2, 3). The expression of 120 of these genes was selectively increased in pancreatic cancer compared to either normal pancreas or CP tissues (fig. 2). In 195 of these genes, expression was increased in both pancreatic cancer and CP when compared to the normal pancreas. In the remaining 152 genes, there was an increase in expression in the cancer compared to normal pancreatic tissues, but not in comparison with the levels observed in the CP samples (fig. 3).
A third group of genes (341 genes, 6.1%) exhibited lower levels of expression in the cancer samples compared to the normal pancreas (tables 4, 5). Ninety-six of these genes were also expressed at low levels compared to the CP tissues (fig. 2), whereas 54 genes were expressed at

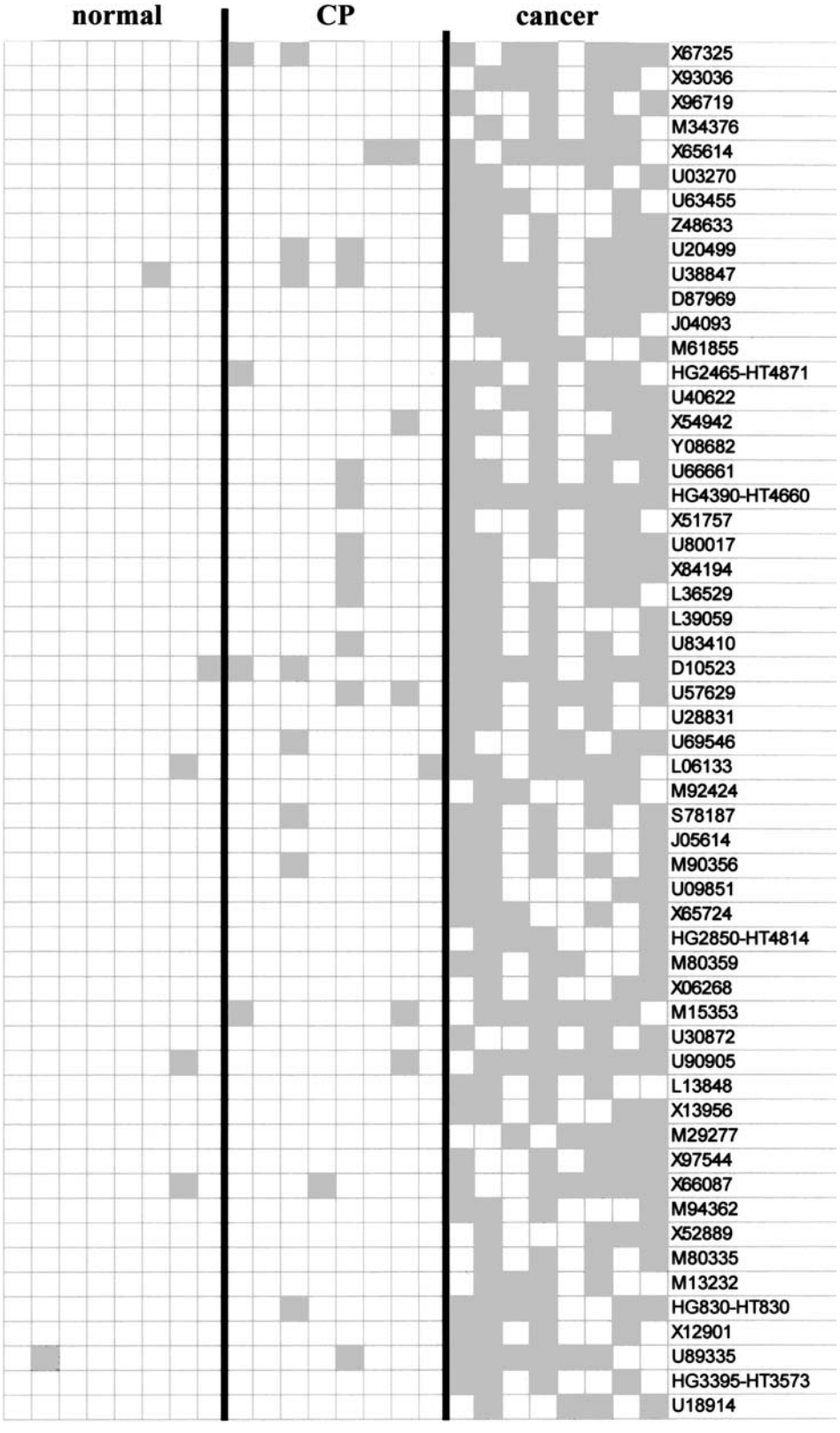

Figure 1. Fifty-six of 5600 genes that were expressed in at least four of eight pancreatic cancer samples but in one or none of the eight normal pancreatic samples. Gray, expression of the gene is detected; white, expression of the gene is below the detection level of the microarray analysis. The GenBank accession numbers are depicted on the right.

Table 1. Pancreatic cancer-specific genes: genes present in at least 50% of the cancer samples but absent in normal pancreatic and CP samples (33 of 5600 genes; 18 genes shown).

No.	Fold *increase*		GenBank accession number	Annotation	Alternative designations
	CA vs NO	CA vs CP			
1	*40.34*	*40.34*	X93036	FXYD domain-containing ion transport regulator 3	phospholemman-like; PLML mammary tumor, 8-kD; MAT-8
2	*13.91*	*7.32*	X96719	C-type lectin superfamily member 2	activation- induced C-type lectin; AICL
3	*12.83*	*12.59*	M34376	β-microseminoprotein	microseminoprotein, beta; MSMB
4	*6.93*	*5.18*	U03270	centrin 1	centrin 1; CETN1
5	*5.47*	*7.15*	U63455	ATP-binding cassette, subfamily C, member 8	sulfonylurea receptor; SUR, SUR1
6	*5.29*	*5.52*	Z48633	retrotransposon	
7	*4.47*	*3.39*	D87969	solute carrier family 35, member 1	cytidine monophosphate-sialic acid transporter; CST, CMP-SIA
8	*4.32*	*5.97*	J04093	UDP glycosyltransferase 1 family, polypeptide A6	
9	*4.29*	*3.03*	M61855	cytochrome P450, subfamily IIC,	cytochrome P450, subfamily IIC, polypeptide 9; CYP2C9
10	*3.78*	*3.77*	U40622	X-ray repair complementing defective repair in Chinese hamster cells 4	XRCC4
11	*3.58*	*3.58*	Y08682	muscle carnitine palmitoyltransferase I	CPT1B
12	*2.85*	*3.08*	X51757	heat shock 70-kDa protein 6	HSPA6
13	*2.45*	*2.66*	L39059	TATA box-binding protein (TBP)-	TBP-associated factor, RNA polymerase I, 110-kD; TAFI10 SL1, 110-kD subunit
14	*2.15*	*1.89*	U28831	human protein immunoreactive with anti-PTH polyclonal antibodies	
15	*1.89*	*3.4*	M92424	mouse double minute 2, human homolog of p53-binding protein	p53-binding protein MDM2; oncoprotein MDM2
16	*1.65*	*2.27*	U09851	zinc finger protein 148	zinc-binding protein 89; ZBP89 HT-beta
17	*1.6*	*2.16*	M80359	MAP/microtubule affinity-regulating kinase 3	CDC25C-associated protein kinase 1; CTAK1
18	*1.56*	*3.25*	X06268	collagen, type II, alpha 1	collagen, type XI, alpha-3; COL11A3

The complete data set of the microarray analysis is available upon request. CA, cancer; NO, normal; CP, chronic pancreatitis.

low levels in both the cancer and CP samples. For the remaining 191 genes, there was no observed decrease in expression in the cancer samples compared to CP tissues, which, in turn, were not decreased when compared with the normal pancreas. Altogether, there was differential expression of 808 genes (14.4%) in pancreatic cancer tissues compared to the normal pancreas, and of 216 genes (3.9%) compared to both normal and CP tissue samples (fig. 3).

Cluster analysis of the expression data revealed that the overall global pattern of expression was able to distinguish cancerous tissues from normal in almost all cases (excluding one cancerous sample cluster in the normal samples). CP samples, however, seemed to have similarities to both normal and cancer expression profiles, and these profiles varied between samples (fig. 4). Comparable findings were observed on the gene level. Thus, cluster analysis identified 110 genes selectively upregulated in pancreatic cancer compared to 120 genes identified by the basic expression analysis. Similarly, cluster analysis identified 108 genes that were selectively downregulated in pancreatic cancer in comparison to 96 specifically downregulated genes identified by the basic expression analysis. Interestingly, cluster analysis also revealed 178 genes whose expression was downregulated in only a subset of tumors, while in the remaining pancreatic cancer samples, the expression was not different from normal and CP tissues (fig. 4).

Table 2. Genes *increased* in pancreatic cancer: 120 genes increased in pancreatic cancer (CA) versus both CP and the normal (NO) pancreas (120 of 5600 genes; 30 genes shown).

No	Fold *increase*					GenBank accession number	Annotation
	CA vs NO	p value	CA vs CP	p value	CP vs NO		
1	*45.5*	0.052	*16.6*	0.046	2.7	L23808	matrix metalloproteinase 12
2	*42.3*	0.112	*17.7*	0.433	2.4	X54667	cystatin S
3	*40.3*	0.007	*40.3*	0.004	1.0	X93036	FXYD-3
4	*37.5*	0.022	*19.2*	0.016	2.0	J05036	cathepsin E
5	*24.3*	0.296	*10.1*	0.291	2.4	Y07755	S100 calcium-binding protein A2
6	*20.6*	0.055	*7.7*	0.064	2.7	U17760	laminin, beta 3
7	*18.0*	0.223	*8.9*	0.217	2.0	K02215	angiotensinogen
8	*14.8*	0.669	*5.6*	0.386	2.7	X57348	stratifin
9	*13.9*	0.175	*7.3*	0.172	1.9	X96719	C-type lectin, superfamily member 2
10	*12.8*	0.154	*12.6*	0.126	1.0	M34376	microseminoprotein, beta
11	*12.8*	0.315	*10.9*	0.288	1.2	X64810	proprotein convertase subtilisin/kexin type 1
12	*12.5*	0.012	*11.4*	0.009	1.1	X53587	integrin, beta 4
13	*12.3*	0.006	*4.6*	0.010	2.7	M68891	GATA-binding protein 2
14	*12.1*	0.053	*7.5*	0.048	1.6	L34155	laminin, alpha 3
15	*11.7*	0.081	*8.4*	0.081	1.4	U31201	laminin, gamma 2
16	*11.5*	0.002	*10.9*	0.001	1.1	U45878	baculoviral IAP repeat-containing 3
17	*11.0*	0.014	*3.8*	0.030	2.9	Y00503	keratin 19
18	*10.8*	0.068	*7.3*	0.063	1.5	X65614	S100 calcium-binding protein P
19	*10.6*	0.001	*9.7*	0.000	1.1	S73885	transcription factor AP-4
20	*10.1*	0.106	*3.4*	0.185	3.0	U89922	lymphotoxin beta (TNF superfamily member 3)
21	*10.0*	0.150	*4.9*	0.162	2.0	U59321	DEAD/H box polypeptide 17
22	*10.0*	0.007	*4.4*	0.014	2.3	AF000424	DNA segment on chromosome 6 (unique) 49
23	*9.2*	0.304	*6.7*	0.298	1.4	X96783	synaptotagmin 5
24	*9.1*	0.002	*3.2*	0.008	2.8	AF001294	tumor suppressing subtransferable candidate 3
25	*9.1*	0.000	*5.5*	0.000	1.7	D86974	KIAA0220 protein
26	*9.1*	0.158	*5.2*	0.168	1.8	AB002409	inducible cytokine subfamily A, member 21
27	*8.5*	0.365	*9.5*	0.579	0.9	X16662	annexin A8
28	*8.4*	0.005	*3.1*	0.020	2.8	M31516	decay accelerating factor for complement
29	*8.4*	0.249	*5.5*	0.270	1.5	X16354	CEA-related cell adhesion molecule 1
30	*8.2*	0.293	*8.7*	0.233	0.9	X12662	arginase, liver

Differences for 64 of the 120 genes were significant at the 0.05 level.The complete data set of the microarray analysis is available upon request.

Using Internet resources as outlined in Materials and methods, we next analyzed the subcellular localization of the proteins encoded by the differentially expressed genes. Approximately 50% of the genes whose expression was increased in the cancer samples encoded proteins that localized either to the cytoplasm (25%) or the membrane (27%). Similarly, approximately 50% of the genes whose expression was decreased in the cancer samples encoded proteins that localized either to the cytoplasm (25%) or the membrane (23%). In general, there was no obvious difference in the percentage of genes whose expression was increased or decreased in relation to the localization of their encoded proteins. The only exception was the localization in mitochondria. Thus, only 0.86% of the gene products of the increased genes localized to this compartment, whereas 3.5% of the products of the decreased genes were localized in the mitochondria.

We next analyzed the potential molecular function of the proteins encoded by the differentially expressed genes, using the Internet resources outlined in Materials and methods. Approximately 21% of the increased genes and 25% of the decreased genes encoded enzymes. Genes encoding cell surface receptors and ligands formed the next most abundant group in this category. Thus, 7.5% of the increased genes encoded cell surface receptors and 3.4% encoded ligands involved in signal transduction. Similarly, 8.2% of the decreased genes encoded signal-transducing receptors, and 5% encoded ligands. Other important groups included nucleic acid-binding (7.9% of the increased genes, 7.3% of the decreased genes) and transporter (3.4% of the increased genes, 7.3% of the decreased genes) proteins. For most of these functional categories, the percentage of genes in the increased and decreased groups was similar. However, cell adhesion proteins (4.9% of the increased genes, 1.8% of the decreased genes), defense/immunity proteins (6% of the increased genes, 2.1% of the decreased genes), and structural proteins (9.2% of the increased genes, 3.5% of the decreased genes) were overrepresented in the increased-genes group.

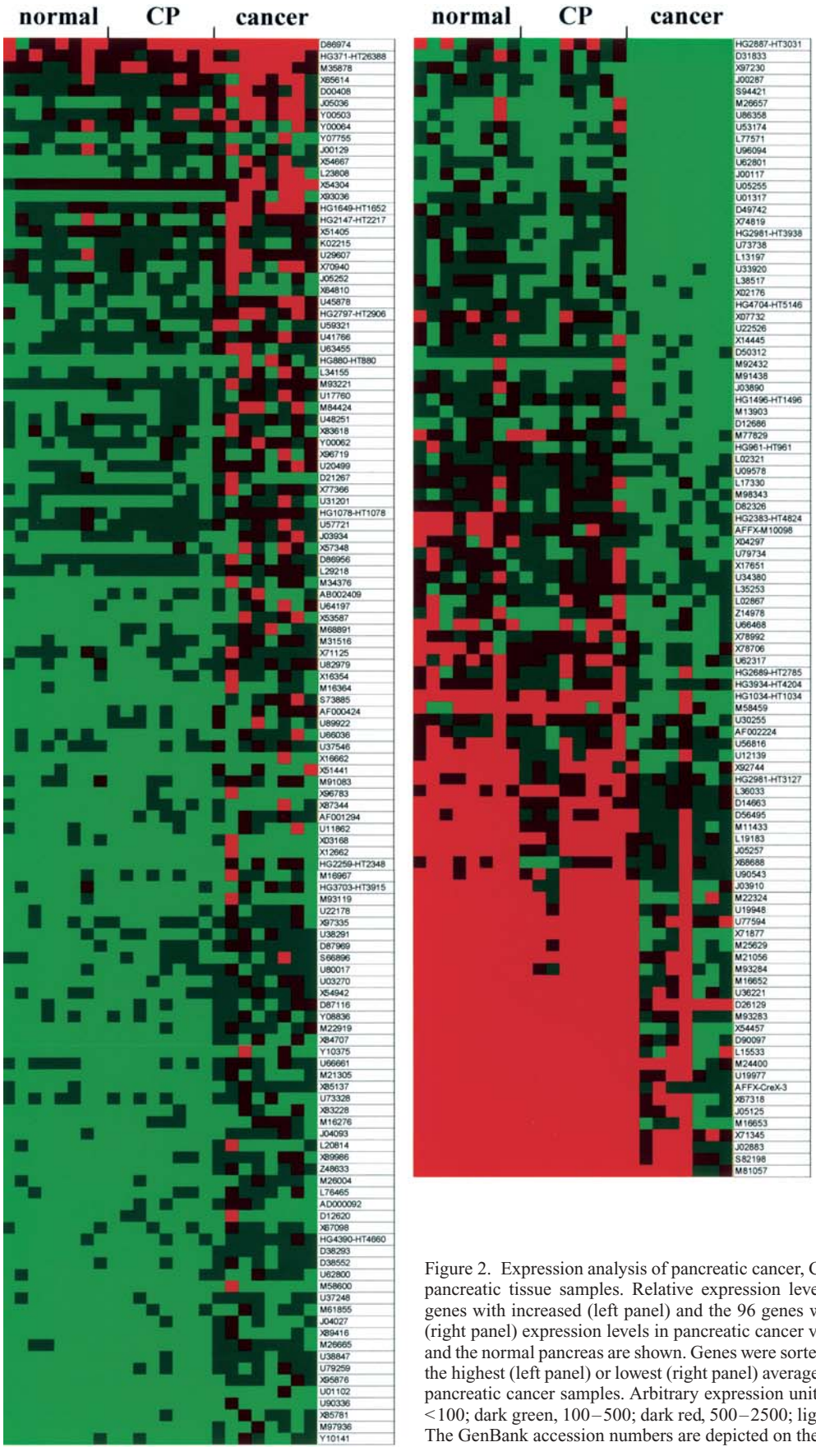

Figure 2. Expression analysis of pancreatic cancer, CP, and normal pancreatic tissue samples. Relative expression levels of the 120 genes with increased (left panel) and the 96 genes with decreased (right panel) expression levels in pancreatic cancer versus both CP and the normal pancreas are shown. Genes were sorted according to the highest (left panel) or lowest (right panel) average expression in pancreatic cancer samples. Arbitrary expression units: light green, <100; dark green, 100–500; dark red, 500–2500; light red, >2500. The GenBank accession numbers are depicted on the right.

Table 3. Genes *increased* in pancreatic cancer: 195 genes increased both in pancreatic cancer (CA) and CP versus the normal (NO) pancreas (195 of 5600 genes; 30 genes shown).

No	Fold *increase*					GenBank accession number	Annotation
	CA vs NO	p value	CA vs CP	CP vs NO	p value		
1	*68.7*	0.019	3.9	*17.6*	0.315	X52003	trefoil factor 1
2	*53.4*	0.006	4.8	*11.1*	0.349	X67325	interferon, alpha-inducible protein 27
3	*52.4*	0.029	7.8	*6.7*	0.777	M29540	CEA-related cell adhesion molecule 5
4	*50.2*	0.014	2.1	*23.6*	0.201	M18728	CEA-related cell adhesion molecule 6
5	*37.2*	0.150	1.3	*29.3*	0.011	Z74616	collagen, type I, alpha 2
6	*35.3*	0.005	2.8	*12.4*	0.303	D13666	osteoblast specific factor 2 (fasciclin I-like)
7	*33.1*	0.056	6.2	*5.4*	0.755	Z19574	keratin 17
8	*22.8*	0.008	1.1	*20.4*	0.023	L13210	lectin, galactoside-binding, 3 binding protein
9	*22.6*	0.014	1.1	*20.0*	0.010	M87789	immunoglobulin heavy constant gamma 3
10	*20.6*	0.003	1.4	*15.2*	0.022	U16306	chondroitin sulfate proteoglycan 2 (versican)
11	*20.4*	0.237	6.4	*3.2*	0.715	Z48314	apomucin
12	*20.2*	0.000	2.9	*7.0*	0.157	Y09836	*H. sapiens* mRNA for 3′ UTR of unknown protein
13	*19.2*	0.089	0.8	*24.9*	0.024	M12759	immunoglobulin J polypeptide
14	*17.8*	0.142	1.8	*9.8*	0.053	X02761	fibronectin 1
15	*17.6*	0.008	1.1	*16.3*	0.015	J03040	secreted protein, acidic, cysteine rich
16	*17.5*	0.096	1.8	*9.7*	0.109	X05610	collagen, type IV, alpha 2
17	*17.3*	0.052	4.5	*3.8*	0.706	S72493	keratin 16
18	*17.3*	0.001	3.0	*5.7*	0.230	J05070	matrix metalloproteinase 9
19	*15.3*	0.013	1.5	*10.5*	0.063	M12125	tropomyosin 2 (beta)
20	*14.9*	0.101	0.7	*21.0*	0.020	M25079	hemoglobin, beta
21	*14.8*	0.036	1.9	*7.9*	0.253	M24766	collagen, type IV, alpha 2
22	*14.3*	0.000	1.7	*8.6*	0.025	U21128	lumican
23	*13.7*	0.229	0.8	*16.9*	0.010	M34516	immunoglobulin lambda-like polypeptide 1
24	*13.2*	0.165	1.2	*10.9*	0.019	X06700	collagen, type III, alpha 1
25	*13.1*	0.001	1.4	*9.2*	0.021	L12350	thrombospondin 2
26	*12.9*	0.137	0.8	*15.9*	0.047	X04828	guanine nucleotide-binding protein (G protein)
27	*12.8*	0.235	1.6	*8.1*	0.007	X68314	glutathione peroxidase 2
28	*12.7*	0.000	3.4	*3.8*	0.240	M19722	feline sarcoma viral (v-fgr) oncogene homolog
29	*12.5*	0.001	1.7	*7.5*	0.057	M65292	H factor (complement)-like 1
30	*12.5*	0.003	0.8	*15.0*	0.001	K03021	plasminogen activator, tissue

Differences for 84 of the 195 genes were significant at the 0.05 level.The complete data set of the microarray analysis is available upon request.

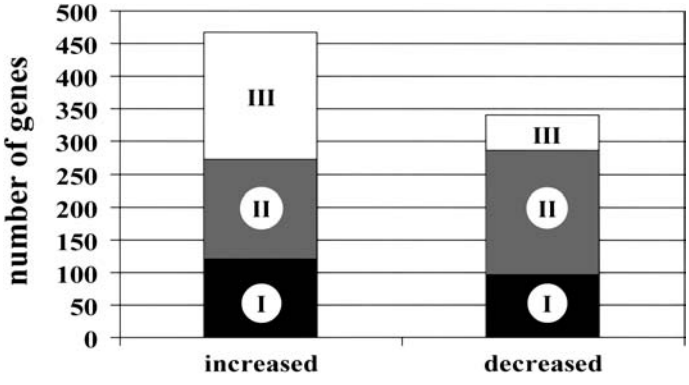

Figure 3. Number of genes whose expression was increased (467/5600) or decreased (341/5600) in pancreatic cancer tissues. Increased or decreased expression was defined as an at least threefold change in expression levels in the cancer tissues compared to normal pancreatic tissues and/or CP tissues. I, genes increased/decreased in pancreatic cancer versus both CP and the normal pancreas; II, genes increased/decreased in pancreatic cancer versus the normal pancreas without changes between cancer and CP as well as CP and the normal pancreas; III, genes increased/decreased in pancreatic cancer and CP versus the normal pancreas.

Table 4. Genes *decreased* in pancreatic cancer: 96 genes decreased in pancreatic cancer (CA) versus both CP and the normal (NO) pancreas (96 of 5600 genes; 30 genes shown).

No	Fold *decrease*					GenBank accession number	Annotation
	CA vs NO	p value	CA vs CP	p value	CP vs NO		
1	*20.0*	0.002	*11.1*	0.060	1.8	X07732	hepsin (transmembrane protease, serine 1)
2	*12.5*	0.000	*8.3*	0.001	1.5	M58459	ribosomal protein S4, Y-linked
3	*12.5*	0.000	*5.6*	0.009	2.2	M25629	kallikrein 1, renal/pancreas/salivary
4	*11.1*	0.001	*4.8*	0.064	2.3	U19948	protein disulfide isomerase
5	*10.0*	0.000	*5.9*	0.005	1.7	X71877	chymotrypsin-like
6	*10.0*	0.000	*5.6*	0.000	1.8	X54457	carboxyl ester lipase (bile salt-stimulated)
7	*10.0*	0.003	*5.0*	0.182	2.0	L38517	Indian hedgehog (*Drosophila*) homolog
8	*9.1*	0.001	*4.8*	0.037	1.9	J05257	dipeptidase 1 (renal)
9	*9.1*	0.000	*3.1*	0.144	2.9	D56495	rat regenerating islet-derived-like
10	*9.1*	0.009	*6.3*	0.113	1.5	D31833	arginine vasopressin receptor 1B
11	*8.3*	0.179	*3.4*	0.501	2.4	X97230	killer cell immunoglobulin-like receptor
12	*8.3*	0.003	*5.0*	0.062	1.7	X92744	defensin, beta 1
13	*8.3*	0.051	*5.9*	0.244	1.4	X14445	fibroblast growth factor 3
14	*8.3*	0.000	*4.3*	0.002	1.9	M93283	pancreatic lipase-related protein 1
15	*8.3*	0.010	*6.7*	0.027	1.2	M77829	aquaporin 1
16	*8.3*	0.000	4.0	0.037	2.1	M22324	alanyl (membrane) aminopeptidase
17	*8.3*	0.000	*5.0*	0.001	1.7	M16652	elastase 1, pancreatic
18	*8.3*	0.008	*4.2*	0.260	2.0	L13197	pregnancy-associated plasma protein A
19	*8.3*	0.087	*5.3*	0.466	1.6	J00287	pepsinogen 5, group I (pepsinogen A)
20	*7.7*	0.018	*3.6*	0.079	2.2	X74819	troponin T2, cardiac
21	*7.7*	0.004	*4.5*	0.154	1.7	U66468	cell growth regulatory with EF-hand domain
22	*7.7*	0.000	*3.1*	0.062	2.5	D14663	KIAA0107 gene product
23	*7.1*	0.001	*3.3*	0.131	2.1	X78706	carnitine acetyltransferase
24	*7.1*	0.000	*4.2*	0.004	1.7	U22526	lanosterol synthase
25	*7.1*	0.000	*3.7*	0.005	1.9	U09578	MAPK-activated protein kinase 3
26	*7.1*	0.007	*5.6*	0.024	1.3	L17330	pre-T/NK cell-associated protein
27	*7.1*	0.000	*4.8*	0.001	1.5	D90097	amylase, alpha 2B; pancreatic
28	*7.1*	0.023	*9.1*	0.001	0.8	D49742	hyaluronan-binding protein 2
29	*6.7*	0.000	*4.0*	0.002	1.7	U36221	glycoprotein 2 (zymogen granule membrane)
30	*6.7*	0.002	*4.0*	0.104	1.7	S94421	TCR eta

Differences for 47 of the 96 genes were significant at the 0.05 level. The complete data set of the microarray analysis is available upon request.

To further validate the data of the microarray analysis, the expression of five genes was analyzed by Northern blotting. This analysis generally confirmed the expression data obtained by microarray analysis. Thus, PSP-94 and MAT-8 were preferentially overexpressed in pancreatic cancer compared to CP and the normal pancreas (fig. 5). Osf-2 and stratifin were also upregulated (fig. 5 and data not shown) and filamin was downregulated (fig. 5) in pancreatic cancer and CP in comparison to normal pancreatic tissues. In addition, Osf-2 and stratifin expression were also evident in cultured pancreatic cancer cell lines as determined by RT-PCR and Northern blot analysis, respectively (fig. 5 and data not shown).

We further analyzed the expression of components of the TGF-β signaling pathway, since this pathway displays frequent alterations in pancreatic cancer, including TGF-β overexpression and Smad4 mutations. The present microarray analysis confirmed the overexpression of TGF-β1 [44] as well as the upregulation of Smad2 [45] and Smad7 [20], which have been previously reported (fig. 6, table 6). Interestingly, our analysis also demonstrated enhanced expression of TAB-1 and TAK-1. TAB-1 is an activator of TAK-1, a member of the mitogen-activated kinase kinase kinase (MAPKKK) family, and is thought to be involved in TGF-β signal transduction [46]. These microarray results were confirmed by Northern blotting, which revealed enhanced expression of TAB-1 and TAK-1 mRNA levels in PDAC tissues in comparison to the normal pancreas (fig. 6). TAB-1 and, to a lesser extent, TAK-1 mRNA moieties were also expressed in cultured pancreatic cancer cells lines (fig. 6).

Discussion

PDACs harbor oncogene and tumor suppressor gene mutations, overexpress mitogenic growth factors and their receptors, and exhibit deregulated expression of growth-inhibiting and apoptosis-modulating molecules [47]. Previous studies of gene expression in PDAC have relied on targeting individual genes for analysis [48]. Recent studies have taken advantage of techniques involving either

Table 5. Genes *decreased* in pancreatic cancer: 54 genes decreased both in pancreatic cancer (CA) and CP versus the normal(NO) pancreas (54 of 5600 genes; 30 genes shown).

No	Fold *decrease*					GenBank accession number	Annotation
	CA vs NO	p value	CA vs CP	CP vs NO	p value		
1	*16.7*	0.053	2.6	*6.3*	0.085	X70083	filamin C, gamma (actin-binding protein-280)
2	*12.5*	0.000	2.7	*4.6*	0.000	M16594	glutathione S-transferase A2
3	*12.5*	0.000	2.2	*5.6*	0.002	U90552	butyrophilin, subfamily 3, member A1
4	*12.5*	0.000	3.4	*3.6*	0.000	X86401	glycine amidinotransferase
5	*11.1*	0.000	1.8	*6.2*	0.001	D17516	AC activating polypeptide 1 receptor type I
6	*9.1*	0.007	2.6	*3.5*	0.029	M15656	aldolase B, fructose-bisphosphate
7	*7.7*	0.000	2.4	*3.2*	0.000	L12711	transketolase
8	*7.7*	0.000	2.1	*3.6*	0.001	M87860	lectin, galactoside-binding, soluble, 2
9	*7.1*	0.000	2.2	*3.2*	0.000	L11005	aldehyde oxidase 1
10	*7.1*	0.044	1.3	*5.5*	0.055	Y08837	X-ray repair complementing defective repair 2
11	*6.7*	0.016	1.4	*4.9*	0.015	M63962	ATPase, H^+/K^+ exchanging, alpha polypeptide
12	*6.3*	0.046	1.0	*6.3*	0.039	D00003	cytochrome P450 (IIIA) polypeptide 3
13	*6.3*	0.047	0.9	*7.1*	0.044	K02268	prodynorphin
14	*5.6*	0.180	1.7	*3.3*	0.255	U81984	endothelial PAS domain protein 1
15	*5.6*	0.000	1.8	*3.1*	0.003	U83908	programmed cell death 4
16	*5.3*	0.129	0.9	*5.6*	0.126	D16626	histidine ammonia-lyase
17	*5.3*	0.206	0.6	*8.3*	0.163	X83863	prostaglandin E receptor 3 (subtype EP3)
18	*5.0*	0.006	1.0	*4.8*	0.009	M24461	surfactant, pulmonary-associated protein B
19	*5.0*	0.084	1.4	*3.5*	0.212	U82818	uncoupling protein 3
20	*4.8*	0.026	1.2	*4.0*	0.034	M25667	growth-associated protein 43
21	*4.8*	0.012	0.6	*7.8*	0.003	M94893	testis-specific protein, Y-linked
22	*4.8*	0.042	1.4	*3.3*	0.084	U12259	paired box gene 3 (Waardenburg syndrome 1)
23	*4.5*	0.061	1.0	4.5	0.058	U82970	phosphate regulating gene
24	*4.5*	0.009	1.1	*4.0*	0.013	V00535	interferon, beta 1, fibroblast
25	*4.3*	0.128	1.1	*4.0*	0.135	U16720	interleukin 10
26	*4.3*	0.038	1.4	*3.0*	0.096	X63755	keratin, cuticle, ultrahigh sulfur 1
27	*4.2*	0.128	1.0	*4.4*	0.146	X97261	metallothionein 1L
28	*4.2*	0.000	1.0	*4.3*	0.000	Z84722	conserved gene telomeric to alpha globin cluster
29	*4.0*	0.189	1.2	*3.4*	0.282	U32376	discs, large (*Drosophila*) homolog 2
30	*3.8*	0.141	1.2	*3.2*	0.191	M37825	fibroblast growth factor 5

Differences for 37 of the 54 genes were significant at the 0.05 level. The complete data set of the microarray analysis is available upon request.

Table 6. Expression levels of members of the TGF-β signaling pathway in pancreatic cancer as compared to the normal pancreas and CP.

Gene	Fold increase	
	cancer vs. normal	cancer vs. CP
TGF-β1	*8.24*	*0.85*
TβRI (ALK5)	0.97	0.91
Smad2	*1.98*	*1.30*
Smad3	1.06	0.82
Smad4	0.91	0.97
Smad7	*2.00*	*1.69*
TAK1	*1.23*	*1.51*
TAB1	*1.34*	*1.27*
p300	1.09	1.04

representational difference analysis (RDA) or microarray analysis [49, 50]. In the latter study, an Affymetrix GeneChip array analysis of 60,000 cDNA fragments identified 180 fragments as having fivefold or greater expression levels in PDAC compared to normal tissue [50]. Of the 124 fragments corresponding to known genes, 107 were identified as differentially expressed in PDAC, and 69 of these genes had not been previously implicated in PDAC [50].

PDAC specimens contain different cell types, including ductal, islet, inflammatory, and nerve cells, ribonuclease-rich acinar cells, and parenchymal elements adjoining the cancer cells exhibit CP-like alterations, including ductal cell proliferation and acinar cell degeneration. There is also an intense stromal reaction in PDAC associated with fibroblast proliferation and collagen deposition. Altogether, the cancer cells represent only 5–10% of the cell population within the pancreatic tumor mass. Utilizing microdissected tissue specimen for microarray analysis would thus be highly interesting. However, obtaining sufficient amounts of high-quality RNA from microdis-

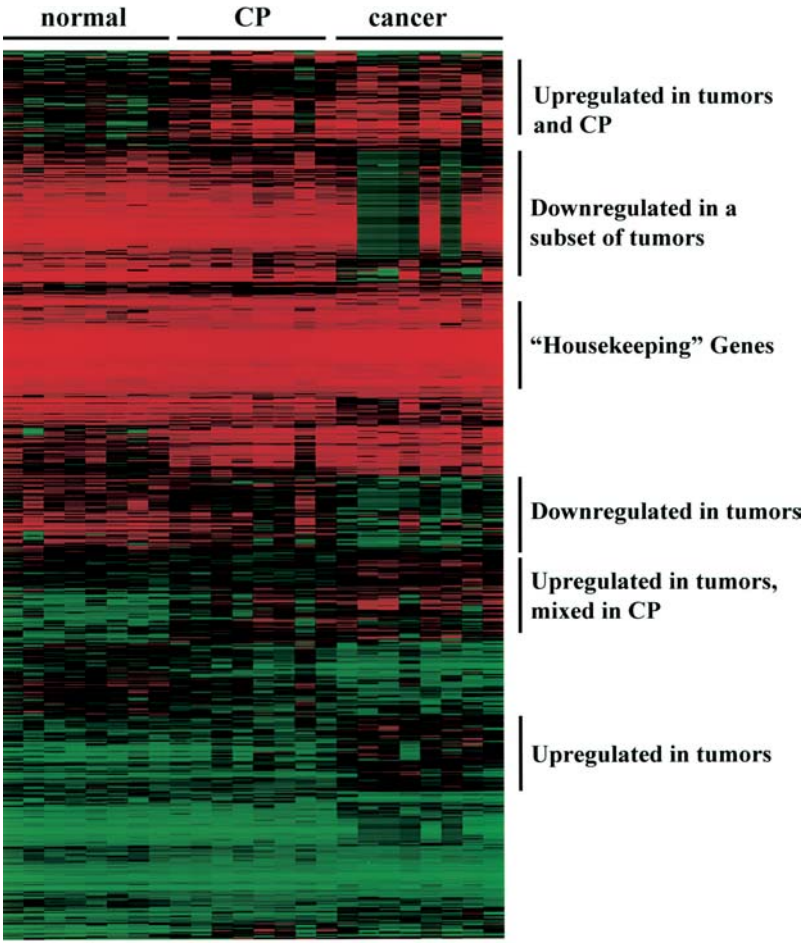

Figure 4. Cluster analysis of the 1336 genes shown to be 'present' in pancreatic cancer, CP, and/or normal pancreatic tissue samples. The coloring of the figure was based on the rank of the expression level compared to other genes on the chip; light red, highest expression; light green, lowest expression.

sected pancreatic tissues is currently extremely demanding technically, in part due to the high RNAse content of this organ. Accordingly, several strategies were used in the present study to assess the global gene expression profile in our samples while avoiding RNA degradation. First, the cancer and CP tissues were macroscopically dissected and snap-frozen in liquid nitrogen in the operating room [51]. Second, frozen-section analysis was used to confirm that samples with abundant cancer cells were utilized for RNA preparation. Third, expression in PDAC tissues was compared with expression in normal and CP tissues in order to delineate alterations in gene expression that are the direct consequence of the malignant phenotype, including alterations that arise from aberrant cancer cell-stromal interactions, but that are distinct from those observed in CP. Utilizing microarrays to analyze the expression of 5600 human genes, we identified 808 (14.4%) differentially expressed genes in PDAC. By comparison with the normal pancreas, the expression of 467 (8.3%) was increased and the expression of 341 genes (6.1%) was decreased. Moreover, 120 genes were upregulated and 96 genes were downregulated in PDAC compared with either the normal pancreas or CP tissues, indicating that microarray analysis of non-microdissected surgical PDAC specimens can be extremely useful for gene profiling and for designing future profiling strategies that will help distinguish between PDAC and CP-associated alterations. However, due to the limited number of samples and multiple testing, some of the differentially expressed genes might have been identified by chance. Therefore, further investigations are necessary to confirm the present findings.

There were several notable observations regarding gene overexpression in the present study. Thus, among the genes that were greatly overexpressed in PDAC in comparison with the normal pancreas and most CP samples, there was increased expression of cystatin S (42-fold), MAT-8 (40-fold), β-microseminoprotein (13-fold), proprotein convertase (PC1/PC3, 13-fold), vitronectin (7-fold), melanoma inhibitory activity (MIA, 7-fold), and

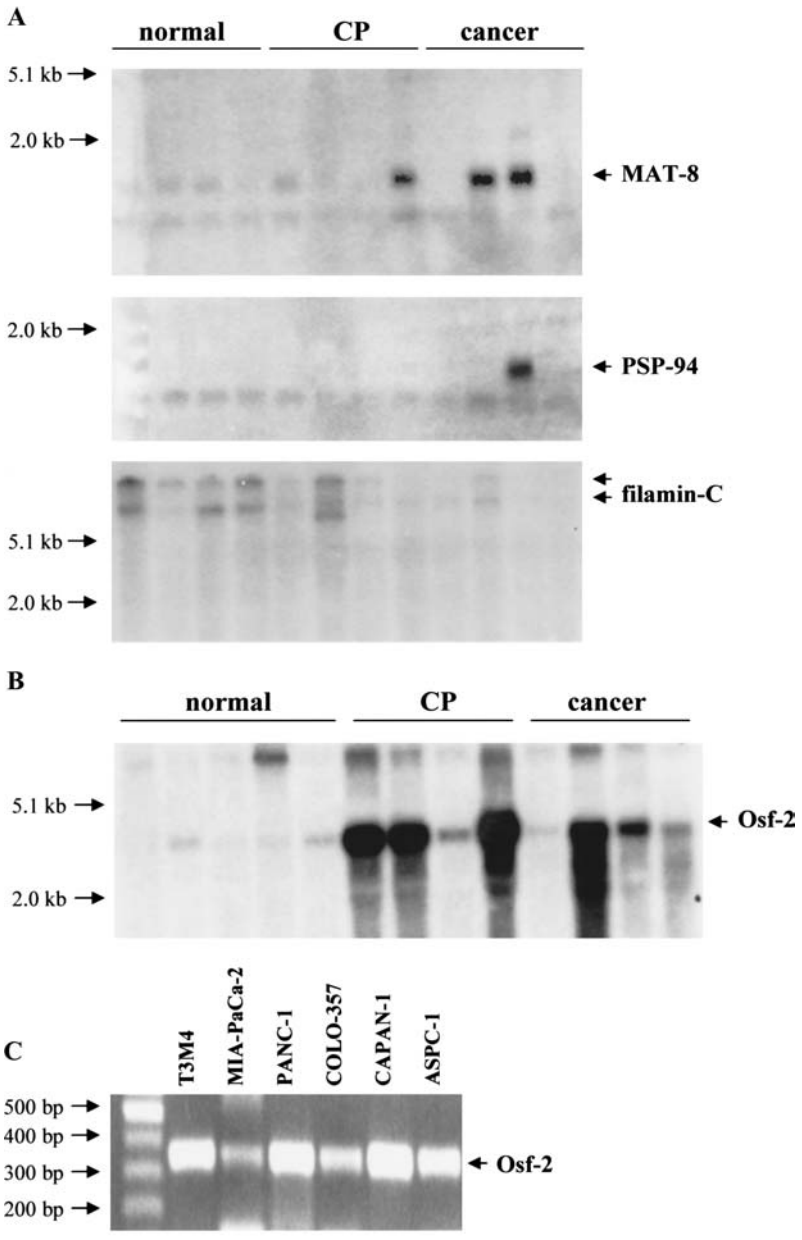

Figure 5. (*A*) Northern blot analysis of MAT-8, PSP-94, and filamin-C mRNA expression in four normal pancreatic, four CP, and four pancreatic cancer tissue samples as described in Materials and methods. The positions of 28S and 18S ribosomal RNA bands are depicted on the left as size markers. (*B*) Northern blot analysis of Osf-2 mRNA expression in five normal pancreatic, four CP, and four pancreatic cancer tissue samples. (*C*) RT-PCR analysis of Osf-2 expression in cultured pancreatic cancer cell lines. The sizes of the DNA ladder are depicted on the left.

centrin 1 (7-fold). None of these genes has been previously reported to be overexpressed in PDAC. Cystatin S belongs to a family of cysteine-proteinase inhibitors [52], and is present in islet cells [53]. Some cystatins, such as cystatin C, have been correlated with suppression of the metastatic phenotype of cancer cells [54], ostensibly by inhibiting the actions of cathepsin B [55]. Other cystatins, such as CMAP, have been implicated in promoting metastasis to the liver [56]. The markedly increased expression of cystatin S observed in the present study therefore raises the possibility that it may contribute to the metastatic potential of pancreatic cancer cells. MAT-8 belongs to the FXYD gene family and encodes an 8-kDa transmembrane protein whose extracellular and transmembrane domains are homologous to the corresponding regions of phospholemman, a major plasmalemmal substrate for cAMP-dependent protein kinase and protein kinase C [57]. It is variably overexpressed in human breast tumors [57], but not to the same extent as noted in PDAC in the current study. Our PDAC samples predominantly ex-

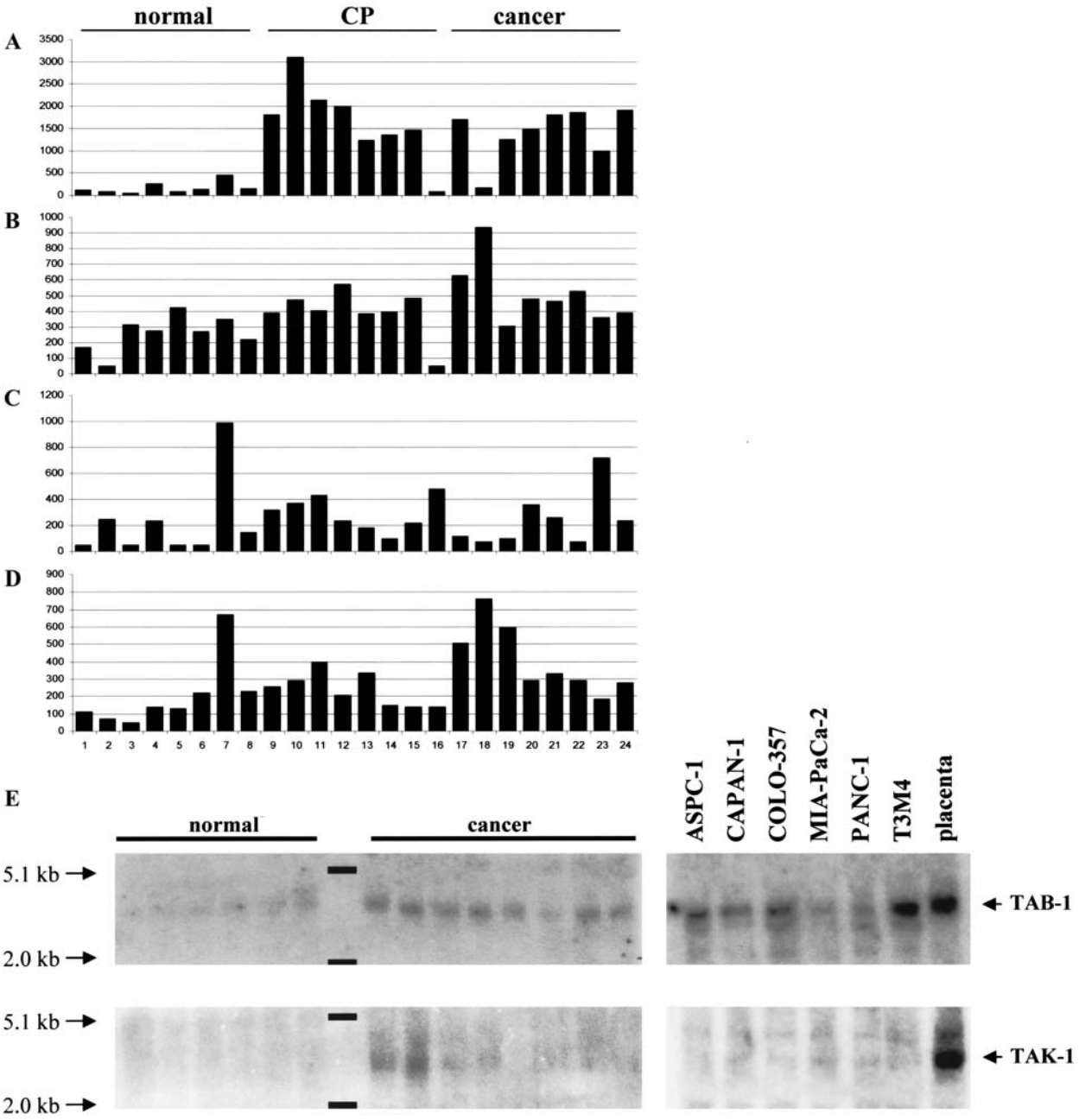

Figure 6. mRNA expression levels of TGF-β1 (*A*), Smad2 (*B*), Smad3 (*C*), and Smad7 (*D*) as determined by microarray analysis in eight normal pancreatic, eight CP, and eight pancreatic cancer tissues. Arbitrary expression levels are depicted on the left. (*E*): Northern blot analysis of TAB-1 and TAK-1 mRNA expression in six normal pancreatic and eight pancreatic cancer samples as well as in six cultured pancreatic cancer cell lines and human placenta, as described in Materials and methods. The positions of 28S and 18S ribosomal RNA bands are depicted on the left as size markers.

pressed high levels of the 1.5-kb MAT-8 transcripts, whereas breast cancers overexpress predominantly the 0.5-kb, and to a lesser extent the 1.5-kb and 3-kb transcripts [57]. MAT-8 has been implicated in chloride ion transport regulation [58], and chloride ion channel blockers suppress glioma cell migration and invasion [59]. Furthermore, in breast epithelial cells, MAT-8 is associated with transformation by Neu (erbB-2) and Ras [60], and K-ras mutations are very common, whereas erbB-2 is often overexpressed in PDAC [10, 61]. These observations raise the possibility that MAT-8 may contribute to the transformed phenotype and invasive capacity of pancreatic cancer cells. β-Microseminoprotein, also known as β-MSP or PSP-94, is a small protein that is overexpressed in prostate cancer [62] and gastric carcinoids [63], and whose increased expression correlates with disease pro-

gression in both malignancies [63, 64]. PC1/PC3 belongs to a family of serine proteases, some of which have been correlated with tumor progression and enhanced metastatic capacity [65]. Vitronectin is a component of the extracellular matrix that has been implicated in modulating cancer cell and endothelial cell motility [66], whereas MIA has been proposed to inhibit tumor cell attachment to the extracellular matrix, thereby promoting the metastasis of melanoma cells [67]. Finally, centrin 1 is a member of a subfamily of the EF-hand superfamily of calcium-binding proteins [68]. It encodes a protein with an approximate M_r of 20,000, and its sequence has four putative calcium-binding domains as defined by the EF-hand consensus [68]. Centrin 1 localizes at the centrosome of interphase cells and redistributes to the region of the spindle poles during mitosis, raising the possibility that it may have a role in centrosome separation at the time of mitosis, and may therefore contribute to the proliferative potential of pancreatic cancer cells. Together, these observations suggest that all seven genes may represent therapeutic targets in PDAC that could be modulated to suppress pancreatic cancer cell proliferation, invasion, and metastasis.

Some of the genes noted to be expressed at high levels in the present study, such as cathepsin E and laminin, are already known to be overexpressed in PDAC. Cathepsin E is an aspartic protease found in the gastrointestinal tract [69], whose enhanced expression in pancreatic cancer [70] and presence in pancreatic juice has been proposed as a diagnostic marker [71]. In the present study, cathepsin E mRNA levels were increased 37-fold and 19-fold in PDAC in comparison to the normal pancreas and CP, respectively, confirming the potential usefulness of an assay for secreted cathepsin. Similarly, pancreatic cancers have been hypothesized to synthesize and deposit laminin-5 in an abnormal manner at the basement membrane, which might facilitate tumor cell migration [72]. Laminin is a heterotrimeric extracellular matrix protein consisting of alpha, beta, and gamma chains. LAMB3 encodes the beta 3 subunit of laminin 5 [73], and we have now shown increased mRNA levels of LAMB3 in PDAC compared to both the normal pancreas (21-fold) and CP tissues (8-fold). The importance of LAMB3 is underscored by the fact that mutations of this gene have been implicated in junctional epidermolysis bullosa [74], and that pancreatic cancer cells expressing an antisense K-ras exhibit suppressed tumorigenicity and downregulation of the LAMB3 [75]. In addition, there was an approximately 12-fold increase in laminin alpha 3 and gamma 2 mRNA levels in the PDAC samples. The current observations, together with the report that laminin gamma 2 and B2 are increased in PDAC [76] and that there is a strong correlation between laminin gamma 2 expression in PDAC and postoperative hepatic metastasis in patients with PDAC [76], strongly suggest that laminin overexpression contributes to the metastatic potential of pancreatic cancer cells in vivo.

Several additional genes that were expressed at high levels in the current study have a role in promoting cancer cell spread and metastasis, but were not previously implicated in PDAC. These include LI-cadherin [77], β4 integrin [78], and annexin A8 [79]. Other genes in this category but previously implicated in PDAC include the chemokine MIP-3-alpha or exodus [80], and matrix metalloproteinase 9 (MMP-9) and 12 (MMP-12). MMPs belong to a family of matrix-degrading enzymes that are important in tissue remodeling and repair, and during development and inflammation [81]. Their deregulated expression has been suggested to promote cancer cell invasion in PDAC and may act to enhance angiogenesis in mouse insulinomas [35, 36, 82]. Based on information from the present microarray analysis, we recently reported that MMP-12 was overexpressed in 25 of 39 PDAC samples, that MMP-12 was present in both the cancer cells and stromal elements in these cancers, and that enhanced expression of MMP-12 correlated with decreased postoperative survival of PDAC patients [83]. This aberrant gene expression profile points to diverse underlying biological mechanisms that promote the metastatic potential of pancreatic cancer cells.

Surprisingly, there was a 2-fold increase in human parathyroid hormone (PTH)-like mRNA in the cancer samples. This hormone has been implicated in oncogenic osteomalacia, a condition not generally associated with PDAC. Although it is distinct from PTH or PTH-related peptide, and does not cause cancer-associated hypercalcemia, the PTH-like peptide is know to induce Osf-2, a protein that may function as an adhesion molecule in bone formation [84] and that may participate in cell adhesion and/or cell communication [85]. Osf-2 mRNA levels were increased 35-fold in PDAC compared to the normal pancreas, and 2.8-fold compared to CP tissues. Furthermore, Osf-2 is known to be induced by bone morphogenetic protein 2 (BMP-2), which is also overexpressed in PDAC [86]. Together, these observations suggest that a previously unrecognized pathway may exist in PDAC that allows for a PTH-like hormone and BMP-2 to induce Osf-2, which may, in turn, have a pathological role in both PDAC and CP.

Two genes that are ostensibly tumor suppressors, S100 calcium-binding protein A2 (S100A2) and stratifin were also markedly overexpressed in PDAC (24- and 15-fold, respectively) by comparison with the corresponding levels in the normal pancreas. Neither of these genes has been previously implicated in PDAC. S100A2 belongs to the S100 family of genes coding for calcium-binding proteins [87]. Its potential role as a tumor suppressor is based on the observation that its expression is decreased in ovarian cancer [88], breast cancer [89], and laryngeal squamous cell carcinoma [90]. S100 calcium-binding

protein P (S100P) was also expressed at high levels (11-fold increase) in the PDAC samples, in agreement with a recent microarray study [50], raising the possibility that certain members of this family may have an important role in PDAC. Furthermore, stratifin is downregulated in breast cancer and in gastric, colorectal, and hepatocellular cancer cell lines, suggesting that it too may also be a tumor suppressor [91, 92]. Stratifin belongs to the 14-3-3 family of proteins, which mediate signal transduction by binding to phosphoserine-containing proteins [93]. Its expression is induced in response to DNA damage, thereby causing cells to arrest in G2 [94]. The fact that two putative tumor suppressor genes are overexpressed in PDAC raises the possibility that both genes may have additional functions beyond acting as tumor suppressors. A similar situation has been reported for ZO-1, a putative tumor suppressor gene that is underexpressed in breast cancer [95] but overexpressed in PDAC [96]. Conversely, there was a 20-fold decrease in hepsin mRNA levels, even though this gene has been reported to be overexpressed in prostate and ovarian cancers [97, 98], and to promote the growth of hepatoma cells [99]. Hepsin belongs to a family of serine proteases, some of which have been correlated with tumor progression and enhanced metastatic capacity [97, 98]. Previously, the serine protease TMPRSS3 has been reported to be overexpressed in PDAC [100]. In the present study, expression of the related protease PC1/PC3 was increased 13-fold in the PDAC samples. Taken together, these observations suggest that the molecular profile of PDAC may be relatively unique in comparison with other cancers, which may allow for the design of a highly specific diagnostic test based on the gene expression profile of this malignancy. Such profiling would be especially important in patients who present with metastatic lesions originating from an unknown primary tumor, which currently constitutes a significant medical dilemma.

With the exception of the mitochondrial compartment, which was overrepresented in the group of genes whose expression was decreased, there were no differences with respect to the subcellular localization between the increased and decreased genes. By contrast, a functional analysis of the deregulated genes revealed that enzymes represented the largest group for both increased and decreased genes. Not surprisingly, in the decreased group, enzymes showed the most dramatic downregulation (tables 4, 5), and comprised mainly pancreatic enzymes, which are produced by acinar cells, such as kallikrein 1, protein disulfide isomerase (PDI), chymotrypsin-like proteinase, and carboxyl ester lipase. Nonetheless, PDI is overexpressed in chronic lymphocytic leukemia and breast cancer [101, 102], underscoring the fact that PDAC exhibits unique alterations in gene expression when compared with other malignancies. In contrast, enzymes that do not contribute to the differentiated functions of the pancreas were overexpressed. These included enzymes such as cystatin S and cathepsin E, and MMPs, as discussed above. These findings underscore the need to a analyze in detail the role of those genes whose expression was found to be altered in PDAC in the present study.

The second largest group of genes that were increased in PDAC encoded cell surface receptors and ligands, which is in agreement with a large body of evidence showing that pancreatic cancers derive a growth advantage from the upregulation of multiple mitogenic growth factors and their receptors [9]. However, the percentage of decreased genes that encode cell surface receptors and ligands was slightly higher than the percentage in the increased group. With a few exceptions (e.g., ALK-5 [14]), downregulation of growth factors and their receptors has not been previously reported in PDAC. The reason for this discrepancy might include difficulties in detecting the expression of those genes or the lack of appreciation of the relevance of these genes in the pathobiology of PDAC. Irrespective of the reasons, a thorough analysis of the downregulated growth factors in PDAC might yield additional interesting results.

There were also several novel observations regarding the underexpression of other groups of genes in PDAC. Thus, there was a 17-fold decrease in filamin c expression in PDAC in comparison with the normal pancreas. Filamin c belongs to a family of large actin-binding proteins that stabilize three-dimensional actin webs, link them to cell membrane, and act by integrating cellular architectural and signaling functions that are essential for cell movement [103]. Recent studies indicate that filamin may interact with caveolin [104], BRCA2 [105], TNF receptor-associated factor 2 (TRAF-2), SEK-1 [106], which mediates activation of stress-activated protein kinase, the calcium sensing receptor, and SHIP-2 [106]. Other than a report describing filamin expression in the connective tissue adjacent to the cancer cells in colorectal cancer [107], the role of filamins and especially filamin c in human malignancies is currently not known. Its marked underexpression in the present study in conjunction with the tumor suppressor role of BRCA2 in PDAC and the known resistance of pancreatic cancer cells to TNF-induced apoptosis [108] raises the novel possibility that loss of filamin c expression may confer an enhanced proliferative capacity and resistance to apoptosis on these cells.

There was also a 12-fold decrease in glutathione S-transferase (GST) A2 expression in PDAC in comparison with the normal pancreas. The large GST gene family encodes enzymes that have been implicated in detoxification pathways that help protect against the deleterious effects of carcinogens [109]. The many GST isozymes derive from at least seven different but related gene families termed α, μ, π, θ, σ, κ, and ζ [110]. Pancreatic GST

isozyme expression is dependent on factors that regulate gene expression and on inherent gene polymorphisms [111]. Analysis of the human pancreas by reverse-phase high-performance liquid chromatography in conjunction with mass spectrometry has revealed 15 to 30-fold variations among individuals with respect to GST A2 protein levels [112]. Recently, a deletion polymorphism in GST-T1 has been associated with an increased risk for pancreatic cancer among heavy cigarette smokers [113]. The marked underexpression of GST-A2 in the present analysis suggests, therefore, that the role of this isozyme in pancreatic cancer susceptibility needs to be carefully examined in population studies and in cigarette smokers.

The risk of developing PDAC is increased 16-fold in CP patients in comparison with the general population [114], and many of the differentially expressed genes exhibited parallel alterations in PDAC and CP. There are several reasons for this similarity in gene expression. First, the pancreas in both conditions exhibits an intense desmoplastic reaction. Second, the pancreatic parenchyma adjacent to the cancer cells harbors CP-like alterations, including acinar cell degeneration that is associated with loss of expression of digestive zymogen enzymes and regions of ductular cell proliferation. Third, in both conditions, there are variable amounts of inflammatory infiltrates that may also contribute to the gene expression profiles observed in the present study. For example, analysis of gene expression in the TGF-β pathway showed increased expression of TGF-β1, Smad2, and Smad7 in PDAC and CP. Together, these results not only increase our confidence in the ability of the microarray technology to confirm previous findings, but also provide an opportunity to expand our knowledge in the perturbations that occur in signaling pathways in PDAC.

Pancreatic intraepithelial neoplasia (PanIN) are currently considered precursor lesions for pancreatic cancer. To identify genetic/epigenetic alterations that are associated with the development of PanINs, and the progression of these lesions to cancer, as well as to metastatic lesions, microdissection of PanINs of different grades to analyze changes in gene expression between these PanINs, primary, and metastatic pancreatic cancer would be highly interesting.

Comparative expression profiling has been informative in many settings, including gene analysis in cancers of the ovary [115–117], lung [118, 119], breast [120, 121], colon [122, 123], prostate [124–127], liver [28, 128], and stomach [129], as well as rhabdomyosarcomas [130], melanomas [131], head and neck cancers [127, 132], and, most recently, PDAC [50]. This technique has also been employed to identify metastasis and chemoresistance-associated genes [133, 134–137]. While the electronic databases and search resources that were utilized in the present investigation provided a great deal of high-quality information, a number of difficulties were encountered in conducting the search. Many of the genes investigated possess numerous synonyms/abbreviations, problematizing an all-encompassing and complete search for each gene. In addition, many of the genes that were identified have not been completely characterized to date, resulting in considerable gaps in the amount of available information with regard to gene function, expression, and subcellular location. Nonetheless, the present study identified 808 differentially expressed genes in pancreatic cancer. Our findings therefore indicate that global expression analysis on surgically obtained tissue specimens can yield highly biologically important and novel results, which is not surprising given the uniformly poor prognosis of PDAC. We now need to further characterize the expression and localization of the respective gene products using a number of different methods to better understand their biological significance, as we recently did with MMP-12 [83]. Furthermore, knowledge gained from the present study will stimulate additional studies that will lead to an improved understanding of the molecular mechanisms underlying pancreatic malignant growth, and that will help delineate new molecular markers for diagnostic, prognostic, and therapeutic use in this disease. Inasmuch as a number of important differences in gene expression have been delineated between PDAC and CP, the present results may also allow for the design of custom arrays that can be utilized to screen a larger number of patients, and that will ultimately be useful for differentiating between normal pancreas, pancreatic cancer, and CP.

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1 Greenlee R. T., Murray T., Bolden S. and Wingo P. A. (2000) Cancer statistics, 2000. CA Cancer J. Clin. **50:** 7–33
2 Warshaw A. L. and Fernandez-del Castillo C. (1992) Pancreatic carcinoma. N. Engl. J. Med. **326:** 455–465
3 Gudjonsson B. (1987) Cancer of the pancreas: 50 years of surgery. Cancer **60:** 2284–2303
4 Gudjonsson B. (1996) Treatment and survival in 13,560 patients with pancreatic cancer, and incidence of the disease, in the west Midlands: an epidemiological study. Br. J. Surg. **83:** 874
5 Abrams R. A. (2001) Adjuvant therapy for pancreatic adenocarcinoma. J. Gastrointest. Surg. **5:** 6–7
6 Permert J., Hafstrom L., Nygren P. and Glimelius B. (2001) A systematic overview of chemotherapy effects in pancreatic cancer. Acta Oncol. **40:** 361–370
7 Sohn T. A., Yeo C. J., Cameron J. L., Koniaris L., Kaushal S., Abrams R. A. et al. (2000) Resected adenocarcinoma of the pancreas – 616 patients: results, outcomes, and prognostic indicators. J. Gastrointest. Surg. **4:** 567–579
8 Hanahan D. and Weinberg R. A. (2000) The hallmarks of cancer. Cell **100:** 57–70
9 Friess H., Lu Z., Graber H. U., Zimmermann A., Adler G., Korc M. et al. (1998) bax, but not bcl-2, influences the prognosis of human pancreatic cancer. Gut **43:** 414–421

10 Longnecker D. S. and Terhune P. G. (1998) What is the true rate of K-ras mutation in carcinoma of the pancreas? Pancreas **17:** 323–324
11 Friess H., Yamanaka Y., Buchler M., Berger H. G., Kobrin M. S., Baldwin R. L. et al. (1993) Enhanced expression of the type II transforming growth factor beta receptor in human pancreatic cancer cells without alteration of type III receptor expression. Cancer Res. **53:** 2704–2707
12 Wagner M., Kleeff J., Friess H., Buchler M. W. and Korc M. (1999) Enhanced expression of the type II transforming growth factor-beta receptor is associated with decreased survival in human pancreatic cancer. Pancreas **19:** 370–376
13 Dunne M. J., Kane C., Shepherd R. M., Sanchez J. A., James R. F., Johnson P. R. et al. (1997) Familial persistent hyperinsulinemic hypoglycemia of infancy and mutations in the sulfonylurea receptor. N. Engl. J. Med. **336:** 703–706
14 Baldwin R. L., Friess H., Yokoyama M., Lopez M. E., Kobrin M. S., Buchler M. W. et al. (1996) Attenuated ALK5 receptor expression in human pancreatic cancer: correlation with resistance to growth inhibition. Int. J. Cancer **67:** 283–288
15 Hahn S. A., Schutte M., Hoque A. T., Moskaluk C. A., Costa L. T. da, Rozenblum E. et al. (1996) DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. Science **271:** 350–353
16 Schwarte-Waldhoff I., Volpert O. V., Bouck N. P., Sipos B., Hahn S. A., Klein-Scory S. et al. (2000) Smad4/DPC4-mediated tumor suppression through suppression of angiogenesis. Proc. Natl. Acad. Sci. USA **97:** 9624–9629
17 Su G. H., Bansal R., Murphy K. M., Montgomery E., Yeo C. J., Hruban R. H. et al. (2001) ACVR1B (ALK4, activin receptor type 1B) gene mutations in pancreatic carcinoma. Proc. Natl. Acad. Sci. USA **98:** 3254–3257
18 Wagner M., Kleeff J., Lopez M. E., Bockman I., Massaque J. and Korc M. (1998) Transfection of the type I TGF-beta receptor restores TGF-beta responsiveness in pancreatic cancer. Int. J. Cancer **78:** 255–260
19 Goggins M., Shekher M., Turnacioglu K., Yeo C. J., Hruban R. H. and Kern S. E. (1998) Genetic alterations of the transforming growth factor beta receptor genes in pancreatic and biliary adenocarcinomas. Cancer Res. **58:** 5329–5332
20 Kleeff J., Ishiwata T., Maruyama H., Friess H., Truong P., Buchler M. W. et al. (1999) The TGF-beta signaling inhibitor Smad7 enhances tumorigenicity in pancreatic cancer. Oncogene **18:** 5363–5372
21 Naumann M., Savitskaia N., Eilert C., Schramm A., Kalthoff H. and Schmiegel W. (1996) Frequent codeletion of p16/MTS1 and p15/MTS2 and genetic alterations in p16/MTS1 in pancreatic tumors. Gastroenterology **110:** 1215–1224
22 Dong M., Nio Y., Tamura K., Song M. M., Guo K. J., Guo R. X. et al. (2000) Ki-ras point mutation and p53 expression in human pancreatic cancer: a comparative study among Chinese, Japanese, and Western patients. Cancer Epidemiol. Biomarkers Prev. **9:** 279–284
23 Friess H., Buchler M. W., Mueller C. and Malfertheiner P. (1998) Immunopathogenesis of chronic pancreatitis. Gastroenterology **115:** 1018–1022
24 Graber H. U., Friess H., Zimmermann A., Korc M., Adler G., Schmid R. et al. (1999) Bak expression and cell death occur in peritumorous tissue but not in pancreatic cancer cells. J. Gastrointest. Surg. **3:** 74–81
25 Gansauge S., Gansauge F., Yang Y., Muller J., Seufferlein T., Ramadani M. et al. (1998) Interleukin 1beta-converting enzyme (caspase-1) is overexpressed in adenocarcinoma of the pancreas. Cancer Res. **58:** 2703–2706
26 Raitano A. B., Scuderi P. and Korc M. (1990) Binding and biological effects of tumor necrosis factor and gamma interferon in human pancreatic carcinoma cells. Pancreas **5:** 267–277
27 Ungefroren H., Voss M., Jansen M., Roeder C., Henne-Bruns D., Kremer B. et al. (1998) Human pancreatic adenocarcinomas express Fas and Fas ligand yet are resistant to Fas-mediated apoptosis. Cancer Res. **58:** 1741–1749
28 Ozawa F., Friess H., Kleeff J., Xu Z. W., Zimmermann A., Sheikh M. S. et al. (2001) Effects and expression of TRAIL and its apoptosis-promoting receptors in human pancreatic cancer. Cancer Lett. **163:** 71–81
29 Buchler P., Conejo-Garcia J. R., Lehmann G., Muller M., Emrich T., Reber H. A. et al. (2001) Real-time quantitative PCR of telomerase mRNA is useful for the differentiation of benign and malignant pancreatic disorders. Pancreas **22:** 331–340
30 Hiyama E., Kodama T., Shinbara K., Iwao T., Itoh M., Hiyama K. et al. (1997) Telomerase activity is detected in pancreatic cancer but not in benign tumors. Cancer Res. **57:** 326–331
31 Tsutsumi M., Tsujiuchi T., Ishikawa O., Majima T., Yoshimoto M., Sasaki Y. et al. (1997) Increased telomerase activities in human pancreatic duct adenocarcinomas. Jpn. J. Cancer Res. **88:** 971–976
32 Itakura J., Ishiwata T., Friess H., Fujii H., Matsumoto Y., Buchler M. W. et al. (1997) Enhanced expression of vascular endothelial growth factor in human pancreatic cancer correlates with local disease progression. Clin. Cancer Res. **3:** 1309–1316
33 Itakura J., Ishiwata T., Shen B., Kornmann M. and Korc M. (2000) Concomitant over-expression of vascular endothelial growth factor and its receptors in pancreatic cancer. Int. J. Cancer **85:** 27–34
34 Seo Y., Baba H., Fukuda T., Takashima M. and Sugimachi K. (2000) High expression of vascular endothelial growth factor is associated with liver metastasis and a poor prognosis for patients with ductal pancreatic adenocarcinoma. Cancer **88:** 2239–2245
35 Bramhall S. R., Neoptolemos J. P., Stamp G. W. and Lemoine N. R. (1997) Imbalance of expression of matrix metalloproteinases (MMPs) and tissue inhibitors of the matrix metalloproteinases (TIMPs) in human pancreatic carcinoma. J. Pathol. **182:** 347–355
36 Ellenrieder V., Alber B., Lacher U., Hendler S. F., Menke A., Boeck W. et al. (2000) Role of MT-MMPs and MMP-2 in pancreatic cancer progression. Int. J. Cancer **85:** 14–20
37 Cantero D., Friess H., Deflorin J., Zimmermann A., Brundler M. A., Riesle E. et al. (1997) Enhanced expression of urokinase plasminogen activator and its receptor in pancreatic carcinoma. Br. J. Cancer **75:** 388–395
38 Koliopanos A., Friess H., Kleeff J., Shi X., Liao Q., Pecker I. et al. (2001) Heparanase expression in primary and metastatic pancreatic cancer. Cancer Res. **61:** 4655–4659
39 Vogelmann R., Kreuser E. D., Adler G. and Lutz M. P. (1999) Integrin alpha6beta1 role in metastatic behavior of human pancreatic carcinoma cells. Int. J. Cancer **80:** 791–795
40 Furuyama H., Arii S., Mori A. and Imamura M. (2000) Role of E-cadherin in peritoneal dissemination of the pancreatic cancer cell line, panc-1, through regulation of cell to cell contact. Cancer Lett. **157:** 201–209
41 Ellenrieder V., Adler G. and Gress T. M. (1999) Invasion and metastasis in pancreatic cancer. Ann. Oncol. **10:** 46–50
42 Wodicka L., Dong H., Mittmann M., Ho M. H. and Lockhart D. J. (1997) Genome-wide expression monitoring in *Saccharomyces cerevisiae*. Nat. Biotechnol. **15:** 1359–1367
43 Eisen M. B., Spellman P. T., Brown P. O. and Botstein D. (1998) Cluster analysis and display of genome-wide expression patterns. Proc. Natl. Acad. Sci. USA **95:** 14863–14868
44 Friess H., Yamanaka Y., Buchler M., Ebert M., Beger H. G., Gold L. I. et al. (1993) Enhanced expression of transforming growth factor beta isoforms in pancreatic cancer correlates with decreased survival. Gastroenterology **105:** 1846–1856
45 Kleeff J., Friess H., Simon P., Susmallian S., Buchler P., Zimmermann A. et al. (1999) Overexpression of Smad2 and colo-

calization with TGF-beta1 in human pancreatic cancer. Dig. Dis. Sci. **44:** 1793–1802
46 Shibuya H., Yamaguchi K., Shirakabe K., Tonegawa A., Gotoh Y., Ueno N. et al. (1996) TAB1: an activator of the TAK1 MAPKKK in TGF-beta signal transduction. Science **272:** 1179–1182
47 Kleeff J., Friess H., Berberat P. O., Martignoni M. E., Z'-Graggen K. and Buchler M. W. (2000) Pancreatic cancer – new aspects of molecular biology research. Swiss Surg. **6:** 231–234
48 Friess H., Kleeff J., Korc M. and Buchler M. W. (1999) Molecular aspects of pancreatic cancer and future perspectives. Dig. Surg. **16:** 281–290
49 Wallrapp C., Muller-Pillasch F., Micha A., Wenger C., Geng M., Solinas-Toldo S. et al. (1999) Novel technology for detection of genomic and transcriptional alterations in pancreatic cancer. Ann. Oncol. **10 (suppl) 4:** 64–68
50 Iacobuzio-Donahue C. A., Maitra A., Shen-Ong G. L., Heek T. van, Ashfaq R., Meyer R. et al. (2002) Discovery of novel tumor markers of pancreatic cancer using global gene expression technology. Am. J. Pathol. **160:** 1239–1249
51 Korc M., Chandrasekar B., Yamanaka Y., Friess H., Buchier M. and Beger H. G. (1992) Overexpression of the epidermal growth factor receptor in human pancreatic cancer is associated with concomitant increases in the levels of epidermal growth factor and transforming growth factor alpha. J. Clin. Invest. **90:** 1352–1360
52 Saitoh E., Isemura S., Sanada K. and Ohnishi K. (1991) The human cystatin gene family: cloning of three members and evolutionary relationship between cystatins and Bowman-Birk type proteinase inhibitors. Biomed. Biochim. Acta **50:** 599–605
53 Lignelid H. and Jacobsson B. (1992) Cystatin C in the human pancreas and gut: an immunohistochemical study of normal and neoplastic tissues. Virchows Arch. A Pathol. Anat. Histopathol. **421:** 491–495
54 Sexton P. S. and Cox J. L. (1997) Inhibition of motility and invasion of B16 melanoma by the overexpression of cystatin C. Melanoma Res. **7:** 97–101
55 Pavlova A., Krupa J. C., Mort J. S., Abrahamson M. and Bjork I. (2000) Cystatin inhibition of cathepsin B requires dislocation of the proteinase occluding loop: demonstration by release of loop anchoring through mutation of his110. FEBS Lett. **487:** 156–160
56 Morita M., Yoshiuchi N., Arakawa H. and Nishimura S. (1999) CMAP: a novel cystatin-like gene involved in liver metastasis. Cancer Res. **59:** 151–158
57 Morrison B. W., Moorman J. R., Kowdley G. C., Kobayashi Y. M., Jones L. R. and Leder P. (1995) Mat-8, a novel phospholemman-like protein expressed in human breast tumors, induces a chloride conductance in *Xenopus* oocytes. J. Biol. Chem. **270:** 2176–2182
58 Sweadner K. J. and Rael E. (2000) The FXYD gene family of small ion transport regulators or channels: cDNA sequence, protein signature sequence, and expression. Genomics **68:** 41–56
59 Soroceanu L., Manning T. J. Jr and Sontheimer H. (1999) Modulation of glioma cell migration and invasion using Cl(–) and K(+) ion channel blockers. J. Neurosci. **19:** 5942–5954
60 Morrison B. W. and Leder P. (1994) neu and ras initiate murine mammary tumors that share genetic markers generally absent in c-myc and int-2-initiated tumors. Oncogene **9:** 3417–3426
61 Yamanaka Y., Friess H., Kobrin M. S., Buchler M., Kunz J., Beger H. G. et al. (1993) Overexpression of HER2/neu oncogene in human pancreatic carcinoma. Hum. Pathol. **24:** 1127–1134
62 Imasato Y., Xuan J. W., Sakai H., Izawa J. I., Saito Y., Chin J. L. et al. (2000) PSP94 expression after androgen deprivation therapy: a comparative study with prostate specific antigen in benign prostate and prostate cancer. J. Urol. **164:** 1819–1824
63 Weiber H., Borch K., Sundler F. and Fernlund P. (1999) Beta-microseminoprotein in gastric carcinoids: a marker of tumour progression. Digestion **60:** 440–448
64 Sakai H., Tsurusaki T., Kanda S., Koji T., Xuan J. W. and Saito Y. (1999) Prognostic significance of beta-microseminoprotein mRNA expression in prostate cancer. Prostate **38:** 278–284
65 Cheng M., Watson P. H., Paterson J. A., Seidah N., Chretien M. and Shiu R. P. (1997) Pro-protein convertase gene expression in human breast cancer. Int. J. Cancer **71:** 966–971
66 Kamiyama F., Maeda T., Yamane T., Li Y. H., Ogukubo O., Otsuka T. et al. (2001) Inhibition of vitronectin-mediated haptotaxis and haptoinvasion of MG-63 cells by domain 5 (D5(H)) of human high-molecular-weight kininogen and identification of a minimal amino acid sequence. Biochem. Biophys. Res. Commun. **288:** 975–980
67 Bosserhoff A. K., Echtenacher B., Hein R. and Buettner R. (2001) Functional role of melanoma inhibitory activity in regulating invasion and metastasis of malignant melanoma cells in vivo. Melanoma Res. **11:** 417–421
68 Errabolu R., Sanders M. A. and Salisbury J. L. (1994) Cloning of a cDNA encoding human centrin, an EF-hand protein of centrosomes and mitotic spindle poles. J. Cell Sci. **107:** 9–16
69 Azuma T., Liu W. G., Vander Laan D. J., Bowcock A. M. and Taggart R. T. (1992) Human gastric cathepsin E gene: multiple transcripts result from alternative polyadenylation of the primary transcripts of a single gene locus at 1q31–q32. J. Biol. Chem. **267:** 1609–1614
70 Azuma T., Hirai M., Ito S., Yamamoto K., Taggart R. T., Matsuba T. et al. (1996) Expression of cathepsin E in pancreas: a possible tumor marker for pancreas, a preliminary report. Int. J. Cancer **67:** 492–497
71 Uno K., Azuma T., Nakajima M., Yasuda K., Hayakumo T., Mukai H. et al. (2000) Clinical significance of cathepsin E in pancreatic juice in the diagnosis of pancreatic ductal adenocarcinoma. J. Gastroenterol. Hepatol. **15:** 1333–1338
72 Tani T., Lumme A., Linnala A., Kivilaakso E., Kiviluoto T., Burgeson R. E. et al. (1997) Pancreatic carcinomas deposit laminin-5, preferably adhere to laminin-5, and migrate on the newly deposited basement membrane. Am. J. Pathol. **151:** 1289–1302
73 Pulkkinen L., Gerecke D. R., Christiano A. M., Wagman D. W., Burgeson R. E. and Uitto J. (1995) Cloning of the beta 3 chain gene (LAMB3) of human laminin 5, a candidate gene in junctional epidermolysis bullosa. Genomics **25:** 192–198
74 Robbins P. B., Lin Q., Goodnough J. B., Tian H., Chen X. and Khavari P. A. (2001) In vivo restoration of laminin 5 beta 3 expression and function in junctional epidermolysis bullosa. Proc. Natl. Acad. Sci. USA **98:** 5193–5198
75 Ohnami S., Matsumoto N., Nakano M., Aoki K., Nagasaki K., Sugimura T. et al. (1999) Identification of genes showing differential expression in antisense K-ras-transduced pancreatic cancer cells with suppressed tumorigenicity. Cancer Res. **59:** 5565–5571
76 Takahashi S., Hasebe T., Oda T., Sasaki S., Kinoshita T., Konishi M. et al. (2002) Cytoplasmic expression of laminin gamma2 chain correlates with postoperative hepatic metastasis and poor prognosis in patients with pancreatic ductal adenocarcinoma. Cancer **94:** 1894–1901
77 Grotzinger C., Kneifel J., Patschan D., Schnoy N., Anagnostopoulos I., Faiss S. et al. (2001) LI-cadherin: a marker of gastric metaplasia and neoplasia. Gut **49:** 73–81
78 Mercurio A. M. and Rabinovitz I. (2001) Towards a mechanistic understanding of tumor invasion – lessons from the alpha6beta 4 integrin. Semin. Cancer Biol. **11:** 129–141
79 Chang K. S., Wang G., Freireich E. J., Daly M., Naylor S. L., Trujillo J. M. et al. (1992) Specific expression of the annexin

VIII gene in acute promyelocytic leukemia. Blood **79:** 1802–1810
80 Kleeff J., Kusama T., Rossi D. L., Ishiwata T., Maruyama H., Friess H. et al. (1999) Detection and localization of Mip-3alpha/LARC/Exodus, a macrophage proinflammatory chemokine, and its CCR6 receptor in human pancreatic cancer. Int. J. Cancer **81:** 650–657
81 Parsons S. L., Watson S. A., Brown P. D., Collins H. M. and Steele R. J. (1997) Matrix metalloproteinases. Br. J. Surg. **84:** 160–166
82 Bergers G., Brekken R., McMahon G., Vu T. H., Itoh T., Tamaki K. et al. (2000) Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat. Cell Biol. **2:** 737–744
83 Balaz P., Friess H., Kondo Y., Zhu Z., Zimmermann A. and Buchler M. W. (2002) Human macrophage metalloelastase worsens the prognosis of pancreatic cancer. Ann. Surg. **235:** 519–527
84 Takeshita S., Kikuno R., Tezuka K. and Amann E. (1993) Osteoblast-specific factor 2: cloning of a putative bone adhesion protein with homology with the insect protein fasciclin I. Biochem. J. **294:** 271–278
85 Sugiura T., Takamatsu H., Kudo A. and Amann E. (1995) Expression and characterization of murine osteoblast-specific factor 2 (OSF-2) in a baculovirus expression system. Protein Express. Purif. **6:** 305–311
86 Kleeff J., Maruyama H., Ishiwata T., Sawhney H., Friess H., Buchler M. W. et al. (1999) Bone morphogenetic protein 2 exerts diverse effects on cell growth in vitro and is expressed in human pancreatic cancer in vivo. Gastroenterology **116:** 1202–1216
87 Engelkamp D., Schafer B. W., Mattei M. G., Erne P. and Heizmann C. W. (1993) Six S100 genes are clustered on human chromosome 1q21: identification of two genes coding for the two previously unreported calcium-binding proteins S100D and S100E. Proc. Natl. Acad. Sci. USA **90:** 6547–6551
88 Hough C. D., Cho K. R., Zonderman A. B., Schwartz D. R. and Morin P. J. (2001) Coordinately up-regulated genes in ovarian cancer. Cancer Res. **61:** 3869–3876
89 Wicki R., Franz C., Scholl F. A., Heizmann C. W. and Schafer B. W. (1997) Repression of the candidate tumor suppressor gene S100A2 in breast cancer is mediated by site-specific hypermethylation. Cell Calcium **22:** 243–254
90 Lauriola L., Michetti F., Maggiano N., Galli J., Cadoni G., Schafer B. W. et al. (2000) Prognostic significance of the Ca(2+) binding protein S100A2 in laryngeal squamous-cell carcinoma. Int. J. Cancer **89:** 345–349
91 Vercoutter-Edouart A. S., Lemoine J., Le Bourhis X., Louis H., Boilly B., Nurcombe V. et al. (2001) Proteomic analysis reveals that 14-3-3sigma is down-regulated in human breast cancer cells. Cancer Res. **61:** 76–80
92 Suzuki H., Itoh F., Toyota M., Kikuchi T., Kakiuchi H. and Imai K. (2000) Inactivation of the 14-3-3 sigma gene is associated with 5′ CpG island hypermethylation in human cancers. Cancer Res. **60:** 4353–4357
93 Yaffe M. B., Rittinger K., Volinia S., Caron P. R., Aitken A., Leffers H. et al. (1997) The structural basis for 14-3-3:phosphopeptide binding specificity. Cell **91:** 961–971
94 Hermeking H., Lengauer C., Polyak K., He T. C., Zhang L., Thiagalingam S. et al. (1997) 14-3-3 sigma is a p53-regulated inhibitor of G2/M progression. Mol. Cell **1:** 3–11
95 Hoover K. B., Liao S. Y. and Bryant P. J. (1998) Loss of the tight junction MAGUK ZO-1 in breast cancer: relationship to glandular differentiation and loss of heterozygosity. Am. J. Pathol. **153:** 1767–1773
96 Kleeff J., Shi X., Bode H. P., Hoover K., Shrikhande S., Bryant P. J. et al. (2001) Altered expression and localization of the tight junction protein ZO-1 in primary and metastatic pancreatic cancer. Pancreas **23:** 259–265
97 Magee J. A., Araki T., Patil S., Ehrig T., True L., Humphrey P. A. et al. (2001) Expression profiling reveals hepsin overexpression in prostate cancer. Cancer Res. **61:** 5692–5696
98 Tanimoto H., Yan Y., Clarke J., Korourian S., Shigemasa K., Parmley T. H. et al. (1997) Hepsin, a cell surface serine protease identified in hepatoma cells, is overexpressed in ovarian cancer. Cancer Res. **57:** 2884–2887
99 Torres-Rosado A., O'Shea K. S., Tsuji A., Chou S. H. and Kurachi K. (1993) Hepsin, a putative cell-surface serine protease, is required for mammalian cell growth. Proc. Natl. Acad. Sci. USA **90:** 7181–7185
100 Wallrapp C., Hahnel S., Muller-Pillasch F., Burghardt B., Iwamura T., Ruthenburger M. et al. (2000) A novel transmembrane serine protease (TMPRSS3) overexpressed in pancreatic cancer. Cancer Res. **60:** 2602–2606
101 Bini L., Magi B., Marzocchi B., Arcuri F., Tripodi S., Cintorino M. et al. (1997) Protein expression profiles in human breast ductal carcinoma and histologically normal tissue. Electrophoresis **18:** 2832–2841
102 Tager M., Kroning H., Thiel U. and Ansorge S. (1997) Membrane-bound proteindisulfide isomerase (PDI) is involved in regulation of surface expression of thiols and drug sensitivity of B-CLL cells. Exp. Hematol. **25:** 601–607
103 Stossel T. P., Condeelis J., Cooley L., Hartwig J. H., Noegel A., Schleicher M. et al. (2001) Filamins as integrators of cell mechanics and signalling. Nat. Rev. Mol. Cell Biol. **2:** 138–145
104 Stahlhut M. and Deurs B. van (2000) Identification of filamin as a novel ligand for caveolin-1: evidence for the organization of caveolin-1-associated membrane domains by the actin cytoskeleton. Mol. Biol. Cell **11:** 325–337
105 Yuan Y. and Shen Z. (2001) Interaction with BRCA2 suggests a role for filamin-1 (hsFLNa) in DNA damage response. J. Biol. Chem. **276:** 48318–48324
106 Marti A., Luo Z., Cunningham C., Ohta Y., Hartwig J., Stossel T. P. et al. (1997) Actin-binding protein-280 binds the stress-activated protein kinase (SAPK) activator SEK-1 and is required for tumor necrosis factor-alpha activation of SAPK in melanoma cells. J. Biol. Chem. **272:** 2620–2628
107 Porter R. M., Holme T. C., Newman E. L., Hopwood D., Wilkinson J. M. and Cuschieri A. (1993) Monoclonal antibodies to cytoskeletal proteins: an immunohistochemical investigation of human colon cancer. J. Pathol. **170:** 435–440
108 Kleeff J., Kornmann M., Sawhney H. and Korc M. (2000) Actinomycin D induces apoptosis and inhibits growth of pancreatic cancer cells. Int. J. Cancer **86:** 399–407
109 Hayes J. D. and Pulford D. J. (1995) The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit. Rev. Biochem. Mol. Biol. **30:** 445–600
110 Strange R. C., Spiteri M. A., Ramachandran S. and Fryer A. A. (2001) Glutathione-S-transferase family of enzymes. Mutat. Res. **482:** 21–26
111 Ulrich A. B., Schmied B. M., Standop J., Schneider M. B., Lawson T. A., Friess H. et al. (2002) Differences in the expression of glutathione S-transferases in normal pancreas, chronic pancreatitis, secondary chronic pancreatitis, and pancreatic cancer. Pancreas **24:** 291–297
112 Coles B. F., Anderson K. E., Doerge D. R., Churchwell M. I., Lang N. P. and Kadlubar F. F. (2000) Quantitative analysis of interindividual variation of glutathione S-transferase expression in human pancreas and the ambiguity of correlating genotype with phenotype. Cancer Res. **60:** 573–579
113 Duell E. J., Holly E. A., Bracci P. M., Liu M., Wiencke J. K. and Kelsey K. T. (2002) A population-based, case-control study of polymorphisms in carcinogen-metabolizing genes, smoking, and pancreatic adenocarcinoma risk. J. Natl. Cancer Inst. **94:** 297–306
114 Lowenfels A. B., Maisonneuve P., Cavallini G., Ammann R. W., Lankisch P. G., Andersen J. R. et al. (1993) Pancreatitis

and the risk of pancreatic cancer. International Pancreatitis Study Group. N. Engl. J. Med. **328:** 1433– 1437
115 Liotta L. and Petricoin E. (2000) Molecular profiling of human cancer. Nat. Rev. Genet. **1:** 48–56
116 Tapper J., Kettunen E., El-Rifai W., Seppala M., Andersson L. C. and Knuutila S. (2001) Changes in gene expression during progression of ovarian carcinoma. Cancer Genet. Cytogenet. **128:** 1–6
117 Ono K., Tanaka T., Tsunoda T., Kitahara O., Kihara C., Okamoto A. et al. (2000) Identification by cDNA microarray of genes involved in ovarian carcinogenesis. Cancer Res. **60:** 5007–5011
118 Hong T. M., Yang P. C., Peck K., Chen J. J., Yang S. C., Chen Y. C. et al. (2000) Profiling the downstream genes of tumor suppressor PTEN in lung cancer cells by complementary DNA microarray. Am. J. Respir. Cell. Mol. Biol. **23:** 355–363
119 Fung L. F., Lo A. K., Yuen P. W., Liu Y., Wang X. H. and Tsao S. W. (2000) Differential gene expression in nasopharyngeal carcinoma cells. Life Sci. **67:** 923–936
120 Barlund M., Forozan F., Kononen J., Bubendorf L., Chen Y., Bittner M. L. et al. (2000) Detecting activation of ribosomal protein S6 kinase by complementary DNA and tissue microarray analysis. J. Natl. Cancer Inst. **92:** 1252–1259
121 Perou C. M., Sorlie T., Eisen M. B., Rijn M. van de, Jeffrey S. S., Rees C. A. et al. (2000) Molecular portraits of human breast tumours. Nature **406:** 747–752
122 Kitahara O., Furukawa Y., Tanaka T., Kihara C., Ono K., Yanagawa R. et al. (2001) Alterations of gene expression during colorectal carcinogenesis revealed by cDNA microarrays after laser-capture microdissection of tumor tissues and normal epithelia. Cancer Res. **61:** 3544–3549
123 Hernandez A., Smith F., Wang Q., Wang X. and Evers B. M. (2000) Assessment of differential gene expression patterns in human colon cancers. Ann. Surg. **232:** 576–585
124 Bull J. H., Ellison G., Patel A., Muir G., Walker M., Underwood M. et al. (2001) Identification of potential diagnostic markers of prostate cancer and prostatic intraepithelial neoplasia using cDNA microarray. Br. J. Cancer **84:** 1512–1519
125 Vaarala M. H., Porvari K., Kyllonen A. and Vihko P. (2000) Differentially expressed genes in two LNCaP prostate cancer cell lines reflecting changes during prostate cancer progression. Lab. Invest. **80:** 1259–1268
126 Elek J., Park K. H. and Narayanan R. (2000) Microarray-based expression profiling in prostate tumors. In Vivo **14:** 173–182
127 Villaret D. B., Wang T., Dillon D., Xu J., Sivam D., Cheever M. A. et al. (2000) Identification of genes overexpressed in head and neck squamous cell carcinoma using a combination of complementary DNA subtraction and microarray analysis. Laryngoscope **110:** 374–381
128 Graveel C. R., Jatkoe T., Madore S. J., Holt A. L. and Farnham P. J. (2001) Expression profiling and identification of novel genes in hepatocellular carcinomas. Oncogene **20:** 2704–2712
129 El-Rifai W., Frierson H. F. Jr, Harper J. C., Powell S. M. and Knuutila S. (2001) Expression profiling of gastric adenocarcinoma using cDNA array. Int. J. Cancer **92:** 832–838
130 Pandita A., Zielenska M., Thorner P., Bayani J., Godbout R., Greenberg M. et al. (1999) Application of comparative genomic hybridization, spectral karyotyping, and microarray analysis in the identification of subtype-specific patterns of genomic changes in rhabdomyosarcoma. Neoplasia **1:** 262–275
131 Su Y. A., Bittner M. L., Chen Y., Tao L., Jiang Y., Zhang Y. et al. (2000) Identification of tumor-suppressor genes using human melanoma cell lines UACC903, UACC903(+6), and SRS3 by comparison of expression profiles. Mol. Carcinog. **28:** 119–127
132 Chan W. P., Fung M. L., Nobiling R. and Leung P. S. (2000) Activation of local renin-angiotensin system by chronic hypoxia in rat pancreas. Mol. Cell. Endocrinol. **160:** 107–114
133 Clark E. A., Golub T. R., Lander E. S. and Hynes R. O. (2000) Genomic analysis of metastasis reveals an essential role for RhoC. Nature **406:** 532–535
134 Chen K. T., Lin J. D., Chao T. C., Hsueh C., Chang C. A., Weng H. F. et al. (2001) Identifying differentially expressed genes associated with metastasis of follicular thyroid cancer by cDNA expression array. Thyroid **11:** 41–46
135 Cho Y. S., Kim M. K., Cheadle C., Neary C., Becker K. G. and Cho-Chung Y. S. (2001) Antisense DNAs as multisite genomic modulators identified by DNA microarray. Proc. Natl. Acad. Sci. USA **98:** 9819–9823
136 Obexer P., Certa U., Kofler R. and Helmberg A. (2001) Expression profiling of glucocorticoid-treated T-ALL cell lines: rapid repression of multiple genes involved in RNA-, protein- and nucleotide synthesis. Oncogene **20:** 4324–4336
137 Kudoh K., Ramanna M., Ravatn R., Elkahloun A. G., Bittner M. L., Meltzer P. S. et al. (2000) Monitoring the expression profiles of doxorubicin-induced and doxorubicin-resistant cancer cells by cDNA microarray. Cancer Res. **60:** 4161–4166