Gene Expression Analysis of Pancreatic Cell Lines Reveals Genes Overexpressed in Pancreatic Cancer

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Key Words

Pancreas · Cancer · Cell line · Primary isolates ·
Microarray · Thymosin · Immunohistochemistry

Abstract

Background: Pancreatic cancer is one of the leading causes of cancer-related death. Using DNA gene expression analysis based on a custom made Affymetrix cancer array, we investigated the expression pattern of both primary and established pancreatic carcinoma cell lines. **Methods:** We analyzed the gene expression of 5 established pancreatic cancer cell lines (AsPC-1, BxPC-3, Capan-1, Capan-2 and HPAF II) and 5 primary isolates, 1 of them derived from benign pancreatic duct cells. **Results:** Out of 1,540 genes which were expressed in at least 3 experiments, we found 122 genes upregulated and 18 downregulated in tumor cell lines compared to benign

cells with a fold change >3. Several of the upregulated genes (like Prefoldin 5, ADAM9 and E-cadherin) have been associated with pancreatic cancer before. The other differentially regulated genes, however, play a so far unknown role in the course of human pancreatic carcinoma. By means of immunohistochemistry we could show that thymosin β -10 (TMSB10), upregulated in tumor cell lines, is expressed in human pancreatic carcinoma, but not in non-neoplastic pancreatic tissue, suggesting a role for TMSB10 in the carcinogenesis of pancreatic carcinoma. **Conclusion:** Using gene expression profiling of pancreatic cell lines we were able to identify genes differentially expressed in pancreatic adenocarcinoma, which might contribute to pancreatic cancer development.

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Introduction

Pancreatic cancer is the fourth leading cause of cancer death in the United States. It can be estimated that annually 28,000 people die from pancreatic cancer [1].

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The incidence has slightly increased over the past decades, being 8.1 for men and 5.4 for women in Germany [2]. About 80% of patients with pancreatic carcinoma present with an irresectable tumor at the time of diagnosis. Chemotherapy and radiation have not been able to increase the survival rate, and curability of the disease strongly depends on early diagnosis. Although several molecular alterations have been associated with pancreatic carcinoma, a much larger number of genes are involved in processes leading to cell transformation and, in consequence, pancreatic cancer.

DNA microarray technology has proven to be a useful tool to screen the differential mRNA expression pattern of normal and carcinoma cells [3–7]. While for other tumor entities a small piece from the tumor can be excised, homogenized and the mRNA extracted to be analyzed, the morphological growth peculiarities of pancreatic carcinoma impede this approach. Due to the characteristic host desmoplastic reaction to the tumor, only a small fraction of the mRNA extracted from carcinomatous tumor will descend from the transformed ductal cells. A majority will represent the expression profile of the surrounding stromal cells. Cell lines can, however, be used as model systems for tumor development and for the analysis of new treatment options. We therefore determined the differential gene expression in pancreatic carcinoma from established tumor cell lines and primary isolates using a custom made Affymetrix GeneChip. Within the set of genes we identified 140 genes as differentially expressed and validated the overexpression of thymosin β -10 (TMSB10) within ductal pancreatic adenocarcinomas by immunohistochemistry.

Materials and Methods

Cell Lines and RNA Preparation

The ATCC (American Type Culture Collection) cell lines AsPC-1 (CRL-1682), BxPC-3 (CRL-1687), Capan-1 (HTB-79), Capan-2 (HTB-80) and HPAF II (CRL-1997) were a gift from Dr. M. Schmitz, Institute of Immunology, University of Dresden.

UKEAC-99 was obtained from a patient who underwent Whipple's operation for papillary carcinoma of intermediate differentiation [8]. PI3 was obtained from a metastasis in the omentum of a patient with pancreatic adenocarcinoma of intermediate differentiation. PI4 was derived from ascites fluid of a patient with pancreatic adenocarcinoma, and PI5 was obtained from a lung metastasis from a patient with pancreatic adenocarcinoma. All cell lines were tested for tumorigenicity and caused pancreatic adenocarcinoma after injection into mice which were histologically confirmed by a pathologist. Chromosomal analysis was performed with all cell lines.

ATCC cells and primary isolates were propagated in RPMI 1640 medium (Gibco) supplemented with 10% fetal bovine serum (Gibco), 2 mM glutamine (Gibco), nonessential amino acids (5 ml/l), and penicillin/streptomycin (10,000 units/ml penicillin and 10 mg/ml streptomycin, 10 ml/l medium; Sigma) and passaged before confluency.

The hPDC was obtained from the main pancreatic duct of a patient undergoing surgery for chronic pancreatitis. The cells were propagated in Keratinocyte SFM supplemented with 5 ng/ml EGF, 50 μ g/ml BPE, 50 μ g/ml gentamycin, 0.25 μ g/ml Fungizone; 500 μ l/60 mm dish (Invitrogen, Karlsruhe, Germany) [9]. All cells were lysed in a guanidine thiocyanate buffer with 2% β -mercaptoethanol added (Promega, Heidelberg, Germany).

Poly A⁺ RNA was prepared with a PolyATtract 1000 kit (Promega) according to the manufacturer's recommendations. For each sample the cDNA synthesis and in vitro transcription were performed according to the Affymetrix user manual using 500 ng of the isolated RNA. In brief, cDNA synthesis was initiated using the Affymetrix T7-oligo-dT promotor-primer combination at 0.1 mM. From this first strand cDNA, the second strand synthesis was generated with internal priming. The in vitro transcription was performed with Ambion's Megascript kit (Ambion, Huntington, UK), as recommended by the manufacturer. During the in vitro transcription, biotin-labeled nucleotides were incorporated into the aRNA. Hybridization and detection of the labeled aRNA on the metg001A GeneChip were performed according to Affymetrix's instructions.

Chip Design and Data Analysis

The custom metg001A GeneChip used in this study consists of 6,117 probe sets resembling 3,023 cDNA fragments. The cDNA fragments were chosen from a data mining approach to characterize the expression of cancers by an electronic Northern blot [10, 11]. In addition genes of signal transduction cascades, which are implicated in cancer development and progression, like the wnt cascade, were selected.

The background was computed as the mean of the 2% darkest feature intensities. After background correction the arrays were normalized by dividing each feature of a given GeneChip by the median value derived from all features of the same GeneChip. To generate the representative expression value we used the 75% quartile of the ranked intensities from the 20 perfect-matching oligonucleotides of a probe set, in an approach similar to that of Irizarry et al. [12]. To distinguish signal from noise we applied the Wilcoxon signed rank test to each probe set. The probe set was labeled as present if the result of the Wilcoxon signed rank was below 0.01.

To determine the relative difference in expression between the tumor cell lines and the benign ductal cells, the mean expression value in the tumor cell lines was calculated and compared to the expression value in the benign ductal cells. Genes were called as differentially expressed when the change fold was above 3 and when the gene was called present in at least 3 cell lines as well. Genes are listed according to the difference ratio.

Complete linkage clustering of the differentially expressed genes was done using the Genecluster/Treeview software package (<http://rana.lbl.gov/EisenSoftware.htm>) using Spearman rank correlation as distance matrix (fig. 1).

Immunohistochemical Staining

Tumor tissue was selected from 55 consecutive cases of pancreatic ductal adenocarcinoma, whereas adenocarcinoma of the ampulla

of Vater or the distal ductus choledochus was not included. Patients were operated on at ages from 34 to 80 years; 27 were male and 28 were female.

Four-micron sections were cut from the formalin-fixed, paraffin-embedded, pancreatic adenocarcinoma tissue. The sections were mounted on superfrost slides (Menzel Gläser, Braunschweig, Germany), dewaxed with xylene and gradually hydrated. Antigen was exposed by heating the sections under high pressure in Tris-EDTA-citrate buffer. The primary goat polyclonal anti-human TMSB10 antibody [13] was diluted 1:500 in PBS containing 2% horse serum (Vector Laboratories, Burlingame, Calif., USA). After incubation for 45 min, the reaction was visualized with a biotinylated anti-goat antibody (5 µg/ml, Vector Laboratories) and avidin-biotin-peroxidase (ABC ELITE, Vector Laboratories). Diaminobenzidine served as chromogen. Afterwards the slides were briefly counterstained with hematoxylin. The primary antibody was omitted for the negative control. Unspecific goat IgG at the same concentration as the specific Ig were used as isotype controls to assure the specificity of the reaction. Staining intensity was evaluated semi-quantitatively as negative, weak, moderate or strong. The final results, however, were recorded as positive or negative staining only.

Results

Out of the 3,023 cDNA fragments interrogated by gene expression profiling 1,540 genes were rated as present in at least 3 cell lines. Difference in expression was rated significant if the ratio was greater than 3 and if the gene was detected as present in at least 3 cell lines as well. Of the 1,540 genes, 122 were upregulated and 18 were downregulated in respect to the benign cells. Seven genes were upregulated and 3 genes downregulated in primary isolates only. One gene was upregulated in ATCC cell lines only and not in primary isolates (table 1).

Using hierarchical clustering, we generated a heat map of the gene expression of the identified genes to visualize the gene expression within each experiment (fig. 1). Genes encoding proteins of known function have been classified according to functional categories (fig. 2). Within these categories we found that genes associated with cellular proliferation are commonly upregulated. Within the 122 genes we identified several genes, such as ADAM9 or E-cadherin, with known function in carcinoma of the pancreas or other tissues (table 2).

Fig. 1. Hierarchical clustering of differentially expressed genes in pancreatic cells. Black squares indicate that the gene was called absent by the software.

Table 1. Genes differentially expressed within the pancreatic cell lines analyzed**A. Genes upregulated in tumor**

Name	Symbol	Unigene ID	MV/hPDC	BxPC-3	AsPC-1	Capan-1	Capan-2	HPAF2	PI1	PI3	PI4	PI5
CDC28 protein kinase regulatory subunit 2	CKS2	Hs.83758	12.11	15.15	6.87	12.88	14.36	9.50	17.58	20.53	9.75	2.32
Thymosin β -10	TMSB10	Hs.76293	12.08	9.47	12.60	8.83	9.40	10.13	10.89	17.13	17.88	12.40
Similar to transmembrane protein AMIGO3	–	Hs.121520	10.13	5.88	6.84	10.03	5.67	3.52	21.84	10.11	8.60	18.69
CSE1 chromosome segregation 1-like (yeast)	CSE1L	Hs.90073	9.59	5.00	8.35	6.89	8.89	6.89	11.91	19.69	13.42	5.27
Capping protein (actin filament) muscle Z-line, α_2	CAPZA2	Hs.75546	9.52	6.19	7.46	8.39	4.58	6.22	16.97	14.90	10.53	10.47
Eukaryotic translation initiation factor 3, subunit 3 γ , 40 kDa	EIF3S3	Hs.58189	9.48	4.16	9.06	6.98	12.16	10.22	14.32	9.85	7.59	11.02
Small nuclear ribonucleoprotein polypeptide G	SNRPG	Hs.77496	9.39	8.33	6.89	10.82	7.54	7.71	11.25	15.11	10.69	6.17
Prefoldin 5	PFDN5	Hs.288856	9.22	6.91	6.33	6.12	6.36	8.18	13.18	14.29	11.37	10.23
Asparagine synthetase	ASNS	Hs.75692	8.65	5.55	4.14	12.45	2.25	7.68	14.78	5.05	7.29	18.68
Calmodulin 1 (phosphorylase kinase, δ)	CALM1	Hs.374441	7.91	4.89	7.19	8.80	9.57	5.45	10.59	9.17	7.83	7.70
Proliferating cell nuclear antigen	PCNA	Hs.78996	7.42	11.41	4.99	5.51	6.65	5.84	8.30	12.16	10.13	1.79
Cyclin-dependent kinase 4	CDK4	Hs.95577	7.35	6.64	4.81	7.07	8.95	7.57	8.43	9.53	7.69	5.46
Dyskeratosis congenita 1, dyskerin	DKC1	Hs.4747	7.24	6.04	6.28	4.94	8.05	3.23	9.70	11.38	9.12	6.39
Zinc finger protein 9 (a cellular retroviral nucleic-acid-binding protein)	ZNF9	Hs.2110	7.23	8.60	5.98	5.98	6.27	5.16	9.26	8.47	6.54	8.80
Complement component 5 receptor 1 (C5a ligand)	C5R1	Hs.2161	7.15	0.43		11.57	1.39	17.72	5.63	12.30	10.67	4.68
NADH dehydrogenase (ubiquinone) 1a subcomplex, 4, 9 kDa	NDUFA4	Hs.50098	7.04	6.38	6.52	6.87	5.27	5.76	6.12	9.48	10.37	6.62
Mitochondrial ribosomal protein L13	MRPL13	Hs.333823	6.93	6.04	6.46	4.39	6.08	5.42	12.74	10.33	6.50	4.40
Profilin 1	PFN1	Hs.408943	6.82	7.48	5.40	4.42	6.04	4.12	7.49	11.64	7.91	6.85
Hypothetical protein MGC40157	MGC40157	Hs.295362	6.67	5.98	5.82	3.44	6.86	8.44	4.14	9.00	6.10	10.26
A disintegrin and metalloproteinase domain 9 (meltrin γ)	ADAM9	Hs.2442	6.67	4.47	7.26	5.72	5.73	10.26	4.50	9.96	6.90	5.21
Finkel-Biskis-Reilly murine sarcoma virus (FBR-MuSV) ubiquitously expressed (fox derived); ribosomal protein S30	FAU	Hs.177415	6.66	6.20	4.77	6.76	4.94	5.37	5.72	9.02	10.68	6.51
PEST-containing nuclear protein	PCNP	Hs.71618	6.61	5.41	5.58	3.49	4.98	3.82	9.36	12.11	9.95	4.78
Epithelial membrane protein 3	EMP3	Hs.9999	6.59	2.73	2.69	1.24	3.08	1.59	8.94	15.04	10.72	13.24
RAD21 homolog (<i>S. pombe</i>)	RAD21	Hs.81848	6.58	6.22	5.27	3.99	5.94	4.27	12.34	8.84	7.38	5.01
Ribosomal protein L10a	RPL10A	Hs.425293	6.50	4.14	4.72	4.85	4.82	6.89	9.70	8.22	7.47	7.72
Isocitrate dehydrogenase 1 (NADP+), soluble	IDH1	Hs.11223	6.27	4.23	5.43	11.53	5.90	5.00	9.31	4.11	4.29	6.62
High-mobility group nucleosomal-binding domain 2	HMG2	Hs.181163	6.18	7.59	5.22	5.45	5.21	4.46	6.86	9.06	7.35	4.43
DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 39	DDX39	Hs.311609	6.14	3.52	4.86	5.49	7.37	2.98	8.26	11.36	7.65	3.78
NADH dehydrogenase (ubiquinone) 1 β subcomplex, 8, 19 kDa	NDUFB8	Hs.198273	5.90	4.88	5.00	4.28	7.50	7.08	6.32	8.96	5.35	3.67
Fibrillarin	FBL	Hs.99853	5.77	6.28	6.84	3.79	4.29	4.88	7.59	7.08	6.91	4.27
Proteasome (prosome, macropain) subunit, α type, 2	PSMA2	Hs.411773	5.54	3.70	3.34	4.43	3.79	4.60	8.39	9.01	7.58	5.02
Hypothetical protein FLJ14668	FLJ14668	Hs.334644	5.51	5.43	4.06	4.64	3.85	4.03	5.54	8.45	8.16	5.40
Neural precursor cell expressed, developmentally downregulated 5	NEDD5	Hs.155595	5.45	4.89	8.06	8.57	5.82	4.46	4.71	4.77	3.69	4.09
Splicing factor 3b, subunit 1, 155 kDa	SF3B1	Hs.334826	5.35	4.15	4.76	5.56	4.28	5.35	7.03	7.03	5.93	4.07
Ras homolog gene family, member A	ARHA	Hs.77273	5.25	2.14	3.96	7.55	4.38	2.56	8.40	7.53	5.10	5.60
Apoptosis-related protein APR-3	APR-3	Hs.9527	5.23	4.00	5.43	5.89	4.29	3.92	6.72	5.99	5.02	5.83
Triosephosphate isomerase 1	TPI1	Hs.83848	5.21	6.16	5.27	4.36	7.60	5.08	5.54	6.73	3.82	2.34
H1 histone family, member 0	H1F0	Hs.226117	5.20	1.46	3.87	4.69	3.14	3.35	7.37	6.39	6.41	10.15
TAF9 RNA polymerase II, TATA-box-binding protein (TBP)-associated Factor, 32 kDa	TAF9	Hs.60679	5.17	3.16	4.53	2.64	4.94	3.43	7.89	5.98	5.22	8.79
Thioredoxin-interacting protein	TXNIP	Hs.179526	5.16	5.30	5.40	3.15	1.65	5.01	3.14	5.57	3.87	13.31
Cytochrome b-245, α polypeptide	CYBA	Hs.68877	5.13	4.53	4.95	5.43	3.89	4.19	4.50	7.07	6.50	5.12
Lymphocyte antigen 6 complex, locus E	LY6E	Hs.77667	5.13	4.96	6.29	3.91	3.70	2.68	6.75	8.91	5.29	3.64
Similar to <i>S. cerevisiae</i> RER1	RER1	Hs.40500	5.03	2.80	5.53	3.29	3.92	3.91	6.53	9.09	5.33	4.90
Ribosomal protein L4	RPL4	Hs.286	5.01	5.27	4.65	4.96	3.55	3.82	4.11	6.39	7.68	4.68
Cathepsin C	CTSC	Hs.10029	5.00	4.53	3.07	2.46	6.92	4.52	7.05	6.78	6.75	2.92
Hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan syndrome)	HPRT1	Hs.82314	4.95	5.13	2.72	3.79	2.94	3.16	8.76	8.08	6.23	3.71
Actin-related protein 2/3 complex, subunit 1B, 41 kDa	ARPC1B	Hs.433506	4.94	2.49	5.62	3.84	2.47	3.24	4.82	7.59	8.92	5.48
Annexin A4	ANXA4	Hs.422986	4.92	1.70	10.68	5.50	1.09	4.99	9.05	5.36	3.63	2.27
Cofilin 1 (nonmuscle)	CFL1	Hs.180370	4.89	4.52	2.86	3.41	4.71	3.64	6.26	9.61	5.48	3.49
Chaperonin-containing TCP1, subunit 5 (ϵ)	CCT5	Hs.1600	4.85	4.45	3.36	5.52	5.02	2.72	5.77	7.00	6.83	2.99
Ribosomal protein L3	RPL3	Hs.119598	4.84	4.86	4.69	4.77	3.63	3.59	4.20	6.55	6.54	4.77
Rho GDP dissociation inhibitor (GDI) β	ARHGDIB	Hs.83656	4.81	5.40	2.16	4.60	3.70	6.08	5.75	5.90	5.88	3.82

Table 1 (continued)

Name	Symbol	Unigene ID	MV/hPDC	BxPC-3	AsPC-1	Capan-1	Capan-2	HPAF2	PI1	PI3	PI4	PI5
Chromosome 15 open reading frame 15	C15orf15	Hs.284162	4.81	4.61	2.24	6.37	2.59	4.94	7.03	4.59	4.61	6.31
unc-84 homolog A (<i>C. elegans</i>)	UNC84A	Hs.381143	4.76	5.66	5.57	4.02	3.36	3.86	5.99	4.03	4.87	5.48
Siah-interacting protein	SIP	Hs.27258	4.73	2.65	3.26	2.76	4.49	3.88	7.11	7.59	6.68	4.17
Ribosomal protein, large, P1	RPLP1	Hs.424299	4.71	5.14	4.60	4.84	3.51	2.83	4.06	6.37	6.48	4.62
CGI-119 protein	CGI-119	Hs.283670	4.70	3.29	4.24	3.48	2.09	6.03	6.65	6.33	5.45	4.70
Programmed cell death 10	PDCD10	Hs.28866	4.53	2.96	2.32	5.44	3.86	4.32	6.17	5.84	4.67	5.15
Adducin 3 (γ)	ADD3	Hs.324470	4.46	3.95	7.51	4.35	3.07	4.37	6.95	3.51	3.68	2.77
Actin-related protein 2/3 complex, subunit 5, 16 kDa	ARPC5	Hs.82425	4.42	3.77	2.89	3.54	3.75	2.64	5.70	7.60	5.97	3.97
Ribosomal protein S13	RPS13	Hs.165590	4.42	4.12	4.18	4.16	3.18	3.27	3.68	5.80	7.21	4.19
Family with sequence similarity 3, member C	FAM3C	Hs.29882	4.33	2.94	2.60	3.84	5.91	3.09	6.01	4.39	4.44	5.77
Eukaryotic translation initiation factor 3, subunit 5 ϵ , 47 kDa	EIF3S5	Hs.381255	4.27	3.21	2.80	3.28	4.30	3.97	5.43	5.50	5.19	4.73
Protein phosphatase 2 (formerly 2A), catalytic subunit, β isoform	PPP2CB	Hs.80350	4.23	3.84	5.62	2.67	3.37	1.72	5.55	6.23	4.23	4.82
HMT1 hnRNP methyltransferase-like 2 (<i>S. cerevisiae</i>)	HRMT1L2	Hs.20521	4.23	4.56	3.58	3.76	3.36	3.78	3.33	5.91	5.11	4.63
Prohibitin	PHB	Hs.75323	4.20	2.42	2.66	6.85	2.70	4.05	4.91	6.41	5.45	2.36
Chaperonin-containing TCP1, subunit 8 (θ)	CCT8	Hs.15071	4.15	3.00	4.41	2.57	4.72	3.59	6.80	4.29	4.21	3.74
Immediate early response 3	IER3	Hs.76095	4.14	1.95	2.29	2.36	2.03	2.23	5.25	7.53	6.24	7.42
Peroxisedoxin 4	PRDX4	Hs.83383	4.10	3.26	5.38	3.07	4.51	3.53	5.58	5.25	3.84	2.43
Sapiens cDNA: FLJ23477 fis, clone HSI15732	–	Hs.145362	4.07	5.20	3.64	2.55	2.87	3.79	3.93	5.78	5.26	3.57
Heterogeneous nuclear ribonucleoprotein K	HNRPK	Hs.129548	4.06	2.55	3.70	3.81	3.83	3.12	5.42	5.42	4.40	4.33
MCM7 minichromosome maintenance deficient 7 (<i>S. cerevisiae</i>)	MCM7	Hs.77152	4.03	4.32	2.58	5.79	3.69	3.74	4.72	4.96	4.27	2.20
–	EST	–	3.95	4.04	4.16	2.30	3.12	2.75	3.64	5.69	5.69	4.15
Membranecofactor protein (CD46, trophoblast-lymphocyte cross-reactive antigen)	MCP	Hs.83532	3.94	4.10	3.10	3.85	4.53	4.52	5.34	3.85	2.85	3.33
Mitochondrial ribosomal protein L42	MRPL42	Hs.112110	3.91	3.17	1.89	4.70	2.65	3.19	5.42	6.00	4.18	3.95
Heat shock 70 kDa protein 8	HSPA8	Hs.180414	3.90	3.46	4.79	2.91	3.39	3.71	4.34	4.66	3.40	4.46
NADH dehydrogenase (ubiquinone) 1 β subcomplex, 9, 22 kDa	NDUFB9	Hs.15977	3.89	2.62	2.74	4.36	2.91	2.76	5.23	5.56	5.12	3.70
Ribosomal protein L7	RPL7	Hs.153	3.85	2.22	3.56	3.62	3.15	3.35	3.66	5.57	5.46	4.10
Dynein, cytoplasmic, light polypeptide 2A	DNCL2A	Hs.100002	3.77	2.36	4.93	2.56	4.09	2.96	2.95	4.57	4.03	5.51
Matrin 3	MATR3	Hs.78825	3.76	2.79	3.79	2.57	2.90	3.21	5.08	5.33	4.36	3.82
Lysosomal-associated protein transmembrane 4a	LAPTM4A	Hs.111894	3.74	2.50	6.00	3.71	3.11	3.68	4.36	3.58	2.94	3.83
KIAA0152 gene product	KIAA0152	Hs.181418	3.73	3.18	4.21	3.72	2.47	2.43	5.42	5.34	4.46	2.34
BUB3 budding uninhibited by benzimidazoles 3 homolog (yeast)	BUB3	Hs.40323	3.72	3.45	1.81	2.30	4.02	4.06	5.36	5.85	4.28	2.34
Tubulin-specific chaperone a	TBCA	Hs.433254	3.71	1.81	2.84	2.14	2.40	2.54	6.13	5.76	5.90	3.83
Ras-related C3 botulinum toxin substrate 1 (rho family, small GTP-binding protein Rac1)	RAC1	Hs.173737	3.67	2.20	3.04	1.96	2.76	1.90	4.90	5.30	5.16	5.76
TERA protein	TERA	Hs.180780	3.66	2.14	2.97	1.48	2.26	3.02	5.27	5.54	4.90	5.33
Farnesyl diphosphate synthase (farnesyl pyrophosphate synthetase, dimethylallyl-transtransferase, geranyltranstransferase)	FDPS	Hs.335918	3.65	3.54	2.24	4.30	6.03	4.63	3.73	2.61	3.00	2.74
Poly(rC)-binding protein 2	PCBP2	Hs.63525	3.64	3.72	3.35	2.38	3.78	4.39	3.71	4.43	3.51	3.46
Cytochrome C oxidase subunit VIc	COX6C	Hs.351875	3.59	3.35	2.74	2.29	3.49	3.27	4.71	5.28	4.39	2.84
APEX nuclease (multifunctional DNA repair enzyme) 1	APEX1	Hs.73722	3.59	2.45	2.12	2.46	3.08	3.70	4.68	5.45	4.77	3.56
ATPase, H ⁺ transporting, lysosomal 21 kDa, V0 subunit c	ATP6V0B	Hs.7476	3.57	3.59	2.85	5.19	5.20	3.01	2.73	4.10	2.70	2.76
–	EST	–	3.57	0.00	1.52	0.00	3.15	5.51	6.73	4.52	4.95	5.73
Hypothetical protein MGC14151	MGC14151	Hs.333414	3.54	2.67	2.53	4.19	3.22	4.41	3.97	4.79	4.51	1.59
Cathepsin B	CTSB	Hs.297939	3.53	2.61	5.75	1.95	2.83	2.87	3.28	4.19	3.80	4.50
Prostatic-binding protein	PBP	Hs.80423	3.52	2.79	3.35	2.71	2.12	3.20	4.40	6.36	4.17	2.57
Stathmin 1/oncoprotein 18	STMN1	Hs.406269	3.51	2.19	3.72	2.57	2.60	1.66	3.58	6.87	6.03	2.38
Activated RNA polymerase II transcription cofactor 4	PC4	Hs.349506	3.50	2.76	1.81	3.25	2.41	3.60	4.04	5.67	4.30	3.69
NADH dehydrogenase (ubiquinone) 1 β subcomplex, 4, 15 kDa	NDUFB4	Hs.227750	3.50	3.24	2.62	3.05	3.98	3.98	4.11	4.17	3.43	2.93
Uncharacterized hypothalamus protein HT007	HT007	Hs.24371	3.50	3.47	1.65	3.87	2.82	3.08	4.95	4.24	4.09	3.33
Proteasome (prosome, macropain) subunit, α type, 6	PSMA6	Hs.410276	3.50	3.66	2.30	4.30	1.58	2.97	3.56	5.09	5.14	2.87
ATPsynthase, H ⁺ transporting, mitochondrial F0 complex, subunit d	ATP5H	Hs.49018	3.46	2.15	2.13	1.96	3.15	3.32	5.50	5.35	4.39	3.17
Barrier to autointegration factor 1	BANF1	Hs.433759	3.44	3.93	2.13	2.66	2.62	3.24	4.55	5.77	3.18	2.91
Stearyl-CoA desaturase (δ -9-desaturase)	SCD	Hs.119597	3.44	5.59	2.76	3.28	3.96	2.82	3.84	3.19	3.00	2.51
Chaperonin-containing TCP1, subunit 6A (ζ)	CCT6A	Hs.82916	3.42	2.73	2.40	3.11	2.81	2.36	4.46	4.98	4.11	3.77

Table 1 (continued)

Name	Symbol	Unigene ID	MV/hPDC	BxPC-3	AsPC-1	Capan-1	Capan-2	HPAF2	PI1	PI3	PI4	PI5
Interferon, α -inducible protein (clone IFI-15K)	G1P2	Hs.432233	3.41	2.95	4.58	3.91	5.27	3.73	2.29	3.22	2.57	2.15
Peroxiredoxin 5	PRDX5	Hs.31731	3.39	3.36	2.17	2.47	2.33	2.85	3.89	5.17	4.12	4.12
ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit f, isoform 2	ATP5J2	Hs.235557	3.36	4.04	1.31	4.50	3.62	3.87	4.48	3.96	2.90	1.59
Ribosomal protein S9	RPS9	Hs.180920	3.33	3.34	3.11	3.23	2.31	2.52	2.68	4.22	5.49	3.05
X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining; Ku autoantigen, 80 kDa)	XRCC	Hs.84981	3.29	1.88	2.91	2.26	3.00	2.26	5.36	5.03	3.90	3.02
Chromosome 20 open reading frame 43	C20orf43	Hs.182281	3.29	3.69	3.15	2.99	3.03	2.89	3.08	4.21	3.15	3.43
Glioma tumor suppressor candidate region gene 2	GLTSCR2	Hs.421907	3.28	2.88	3.91	1.89	2.42	2.68	3.55	3.42	4.19	4.55
Small nuclear ribonucleoprotein D2 polypeptide 16.5 kDa	SNRPD2	Hs.424327	3.25	3.27	3.06	3.13	2.35	2.59	2.76	4.24	5.06	2.83
Defender against cell death 1	DAD1	Hs.82890	3.24	3.09	2.87	2.70	3.13	3.59	3.98	3.95	2.59	3.30
Protein disulfide isomerase-related protein P5	P5	Hs.182429	3.24	1.98	3.73	2.83	2.80	3.16	4.71	4.27	3.04	2.67
Cell death-regulatory protein GRIM19	GRIM19	Hs.279574	3.23	2.39	2.75	2.88	2.60	2.19	4.07	6.15	3.45	2.60
Death-associated protein 3	DAP3	Hs.159627	3.17	2.19	1.52	2.54	2.98	2.54	3.42	5.04	4.62	3.71
Inorganic pyrophosphatase 2	PPA2	Hs.5123	3.17	3.79	1.86	3.20	2.56	2.93	4.30	4.06	3.93	1.89
Poly(A)-binding protein, cytoplasmic 1	PABPC1	Hs.172182	3.16	2.73	2.82	2.81	3.30	2.72	3.96	3.17	3.24	3.65
Ribosomal protein L13	RPL13	Hs.431392	3.14	3.33	2.95	3.13	2.24	2.44	2.59	4.08	4.56	2.96
Guanine-nucleotide-binding protein (G protein), γ_{12}	GNG12	Hs.8107	3.13	2.81	3.22	2.23	2.40	3.71	4.11	3.53	3.32	2.84
CDC10 cell division cycle 10 homolog (<i>S. cerevisiae</i>)	CDC10	Hs.184326	3.10	2.68	2.38	1.95	2.42	2.45	4.84	4.53	3.85	2.82
Anaplastic lymphoma kinase (Ki-1)	ALK	Hs.9614	3.10	2.49	2.67	2.21	2.49	2.51	3.80	4.89	4.09	2.77

B. Genes downregulated in tumor cell lines

Name	Symbol	Unigene ID	MV/hPDC	BxPC-3	AsPC-1	Capan-1	Capan-2	HPAF2	PI1	PI3	PI4	PI5
Thrombospondin 1	THBS1	Hs.87409	0.31	0.60	0.10	0.13	0.52		0.29	0.30	0.22	0.33
Leucine-rich repeat-containing 8	LRRC8	Hs.173484	0.31	0.51	0.22		0.48	0.20	0.24	0.25	0.29	0.29
Phosphoprotein enriched in astrocytes 15	PEA15	Hs.194673	0.29	0.24	0.19	0.26	0.32	0.21	0.21	0.38	0.40	0.40
Coatmer protein complex, subunit α	COPA	Hs.75887	0.29	0.25	0.25	0.32	0.24	0.30	0.32	0.32	0.31	0.31
Sapiens cDNA FLJ11812 fis, clone HEMBA1006364		Hs.23837	0.28	0.21		0.28	0.49	0.29	0.21	0.26	0.28	0.21
Ets homologous factor	EHF	Hs.182339	0.28	0.31	0.26	0.26	0.20	0.32	0.27	0.30	0.29	0.30
Dual specificity phosphatase 1	DUSP1	Hs.171695	0.27	0.11	0.28	0.09	0.10	0.10	0.16	0.21	0.41	0.94
Tubulin, α_3	TUBA3	Hs.433394	0.25			0.12	0.12		0.10	0.35	0.37	0.42
Small nuclear ribonucleoprotein polypeptide N	SNRPN	Hs.48375	0.24	0.22	0.12	0.50	0.33	0.13	0.15			
Zinc finger protein 217	ZNF217	Hs.155040	0.23	0.24	0.24	0.24	0.14	0.20	0.24	0.25		0.31
Hypothetical protein BM-002	BM-002	Hs.367646	0.23	0.32	0.09	0.21	0.24	0.22				0.29
Amphiregulin (schwannoma-derived growth factor)	AREG	Hs.270833	0.20	0.24		0.21	0.14	0.16	0.20		0.18	0.24
Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	CDKN1A	Hs.179665	0.17	0.16	0.12	0.14	0.14	0.10	0.08	0.19	0.21	0.39
CCR4-NOT transcription complex, subunit 7	CNOT7	Hs.380963	0.17	0.19	0.12	0.13	0.18	0.15	0.16	0.22	0.18	0.15
Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 2	SERPINB2	Hs.75716	0.13	0.71	0.03		0.03		0.05	0.03	0.04	0.04
Serum amyloid A1	SAA1	Hs.332053	0.12	0.10		0.11	0.15					
Hypothetical protein FLJ10154	FLJ10154	Hs.179972	0.10	0.10	0.07	0.07	0.06	0.06	0.12	0.17	0.16	0.10
KIAA0669 gene product	KIAA0669	Hs.52526	0.07		0.04		0.05	0.05	0.07	0.07	0.07	0.10

C. Genes upregulated in primary isolates only

Name	Symbol	Unigene ID	MV all/hPDC	MV ATCC/hPDC	MV PI/hPDC	BxPC-3	AsPC-1	Capan-1	Capan-2	HPAF	PI1	PI3	PI4	PI5
Importin 7	IPO7	Hs.5151	2.31	1.51	3.11		1.90	1.25	1.25	1.63	3.33	3.22	3.37	2.52
Hypothetical protein DKFZp434L142	DKFZp434L142	Hs.323583	2.23	0.95	3.52	0.81	0.95	1.16		0.87	4.94	4.68	3.37	1.08
Transmembrane 4 superfamily member 7	TM4SF7	Hs.26518	3.12	1.90	4.03	1.86	2.07			1.76	2.31	5.57	4.04	4.20
Insulin-like-growth-factor-binding protein 6	IGFBP6	Hs.274313	3.34	2.18	4.50	5.89		0.87	0.81	1.15	5.44	4.80	3.74	4.02
Chromosome 8 open reading frame 4	C8orf4	Hs.283683	3.32	0.88	4.53	0.43		1.34			1.07	2.96	3.62	10.49
Insulin-like-growth-factor-binding protein 3	IGFBP3	Hs.77326	5.29	1.68	8.89		2.87	1.93	1.08	0.84	6.25	9.80	12.39	7.12
Vimentin	VIM	Hs.297753	5.21	1.25	9.17	0.34	3.70		0.63	0.34	6.60	10.34	12.25	7.51

Table 1 (continued)**D. Genes downregulated in primary isolates only**

Name	Symbol	Unigene ID	MV all/ hPDC	MV ATCC/ hPDC	MV PI/ hPDC	BxPC-3	AsPC-1	Capan-1	Capan-2	HPAF2	PI1	PI3	PI4	PI5
CD24 antigen	CD24	Hs.375108	0.41	0.59	0.03	0.70	0.38	0.89		0.40	0.03	0.03	0.02	
Secretory leukocyte protease Inhibitor (antileukoproteinase)	SLPI	Hs.251754	0.56	0.79	0.28	0.79	0.94	1.07	0.41	0.71	0.56	0.15	0.13	0.27
Cadherin 1, E-cadherin	CDH1	Hs.194657	0.58	0.83	0.28	1.11	0.65	1.00	0.79	0.59	0.49	0.17	0.20	0.25

E. Genes upregulated in ATCC cell lines only

Name	Symbol	Unigene ID	MV all/ hPDC	MV ATCC/ hPDC	MV PI/ hPDC	BxPC-3	AsPC-1	Capan-1	Capan-2	HPAF2	PI1	PI3	PI4	PI5
Frizzled 6	FZD6	Hs.114218	3.12	3.55	1.25	6.62	3.97	3.90	3.88	3.75	1.64	0.87	1.11	1.37

The fold change of expression of the tumor cell lines vs. the cell line hPDC. MV/hPDC = fold change of the mean of the expression value of the tumor cell lines vs. hPDC.

Fig. 2. Functional categories of differentially expressed genes. Numbers above the bars indicate the number of genes within the group.

Overexpression of TMSB10 in Human Pancreatic Carcinoma

Expression changes observed in cell lines are not necessarily represented in primary tissues also. Therefore, out of the 122 upregulated genes we selected TMSB10 to analyze the expression of its protein in human pancreatic adenocarcinoma by using a polyclonal antibody and immunohistochemistry. Moreover, TMSB10 is associated with cellular proliferation in a number of cancers indicating that overexpression of this gene might be a causal event [13].

TMSB10 expression was detected in endothelium, smooth muscle cells, neuronal cells, and strongly in macrophages, both in tumor and in normal tissues. In 49 of the 54 (90.7%) ductal adenocarcinomas examined, immunoreactivity for TMSB10 was detected in the tumor cells (fig. 3). Conversely, in none of the 10 sections of normal pancreatic tissue could TMSB10 be shown by means of immunohistochemistry. In 24 of 54 (44.4%) cases, we detected staining in the stroma. The correlation between staining in the tumor and staining in stroma cells, which

Fig. 3. Immunohistochemical staining of non-neoplastic pancreas (**A**), ductal pancreatic adenocarcinoma (**B**) and stromal tissue within a pancreatic adenocarcinoma (**C**) using TMSB10 antibody. Examples for normal ducts (d), endothelial cells (e) and stromal cells (s) are indicated with arrows. The pancreatic adenocarcinoma **B** was scored as 2+ and the sample C as 3+. **A, B** 100 ×, **C** 200 × magnification. **D–F** Strong (**D**), moderate (**E**) and weak (**F**) staining of pancreatic adenocarcinoma samples.

Table 2. Genes known to be involved in tumorigenesis in various tissues

Name	Symbol	Unigene ID	Function	Involved in cancer	Involved in pancreatic cancer	Reference
Prefoldin 5	PFDN5	Hs.288856	Chaperone		Yes	[25]
Thymosin β -10	TMSB10	Hs.76293	Proliferation	Yes		[13, 22, 23]
Calmodulin 1	CALM1	Hs.374441	Signal transduction	Yes	No	[26]
Cellular apoptosis susceptibility protein CSE1L/CAS	CSE1L	Hs.90073	Proliferation and apoptosis	Yes	No	[27]
Lymphocyte antigen 6	LY6E	Hs.77667	Signal transduction	Yes	No	[28]
Cyclin-dependent kinase 4	CDK4	Hs.95577	Cell cycle	Yes	No	[29]
ADAM9	ADAM9	Hs.2442	Proliferation	Yes	Yes	[30]
Prohibitin	PHB	Hs.75323	Proliferation	Yes	No	[31]
Cyclin-dependent kinase inhibitor 1	CDKN1A	Hs.179665	Apoptosis	Yes	No	[32]
E-cadherin	CDH1	Hs.194657	Cell adhesion	Yes	Yes	[16, 18, 33, 34]

Table 3. TMSB10 protein expression in pancreatic ductal adenocarcinoma cells (n = 55) and in stroma cells within the tumors

	Tumor positive	Tumor negative
Stroma positive	20	2
Stroma negative	28	5

p = 0.411, Fisher's exact test.

was not significant, is shown in table 3. Furthermore, we could not find a significant correlation between the positivity or the intensity of expression of TMSB10 and post-operative survival of patients. However, we observed that TMSB10 is highly expressed within well- and poorly-differentiated carcinomas (G1 and G3), whereas the majority of moderately differentiated tumors (G2) expressed TMSB10 only moderately.

Discussion

The DNA microarray technology is widely used as a powerful tool to investigate the mRNA expression pattern of various tissues and cell types. For tissues that are difficult to access or scarcely available, cell lines are a helpful alternative. This is especially true for pancreatic cancer. It is well known that cells change both their morphology and their expression pattern when isolated from their parent organism, the human body [14, 15]. However, analysis of cell lines might circumvent the need for microdissection of tissues and, furthermore, allow the characterization of the most adequate model system for further analysis of differentially expressed genes.

Upregulation of growth factors, cytokines, receptors, extracellular matrix proteins and numerous other proteins is a common event in carcinogenesis. We corroborated this in the pancreatic tumor cell lines analyzed in this study, finding 122 genes upregulated and only 18 downregulated in respect to benign cells.

Apart from the genes differentially regulated in all cell lines, we found 7 genes upregulated and 3 genes downregulated in the primary isolates only, while 1 gene, *frizzled 6*, was upregulated only in the ATCC cell lines. E-cadherin, 1 of the 3 genes downregulated in primary cells, is the main adhesion molecule of epithelia, and it has been implicated in carcinogenesis because it is frequently lost in human epithelial cancers [16]. It is known to act as a strong invasion suppressor in experimental tumor cell systems [17]. Partial or complete loss of E-cadherin expression correlates with malignancy in human cancers [18]. Interestingly, we found downregulation in the primary isolates, but not in the ATCC cells, which may support the assumption that primary cell lines reflect the biological features of the tumor more than established cell lines.

The mesenchymal intermediate filament marker vimentin is strongly expressed in invasive pancreatic carcinomas [19] and generally expressed in pancreatic carcinoma cell lines [20]. The overexpression in primary isolates suggests a relative importance of this filament in cells adapting to the new environment petridish; it seems to be of less importance after adjustment to the different needs of the cells in vitro. Most genes, however, show a comparable degree of expression. This might support the assumption that cells adapt very quickly when isolated and transferred into medium. If they would not, they could not replicate and survive. Within the large set of genes implied in cellular proliferation and identified by the GeneChip analysis we were especially interested in the analysis of TMSB10. The β -thymosins are a family of

related peptides known to be present in a wide variety of mammalian and other vertebrate cells and tissues. TMSB10 mRNA is overexpressed in germ cell tumors of different histological types, breast-, ovarian-, uterine carcinomas, colon, thyroid and esophageal carcinoma cell lines [13, 21, 22]. It has also been shown that TMSB10 plays a critical role in the regulation of anchorage-independent growth and assembly of actin filaments [23]. Furthermore upregulation of TMSB10 was identified in the breast cancer cell line MCF-7 in the course of treatment with 5-fluorouracil, tomudex and oxaliplatin, respectively. TMSB10 expression is also elevated in the 5-fluorouracil-resistant colon cancer cell line H630R10 [24] implying a role for TMSB10 within the reaction of cells on toxic compounds.

In conclusion we have reported for the first time the expression of TMSB10 in pancreatic carcinoma. TMSB10 might play a general role in human carcinogenesis and in pancreatic carcinomas especially. Our finding that TMSB10 is strongly expressed in stroma cells of pancreatic carcinoma supports the suggestion of Santelli et al. [23] who claim a critical role for TMSB10 in the regulation of anchorage-independent growth and assembly of actin filaments.

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