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DNA Microarray Analysis of Pancreatic Malignancies

Regine Brandt[a] Robert Grützmann[b] Andrea Bauer[c] Ralf Jesnowski[a,d]
Jörg Ringel[a] Matthias Löhr[a,d] Christian Pilarsky[b] Jörg D. Hoheisel[c]

[a]Department of Medicine II, Mannheim Medical Faculty, University of Heidelberg; [b]Department of Visceral, Thoracic and Vascular Surgery, University Hospital Carl Gustav Carus, Technical University of Dresden; [c]Division of Functional Genome Analysis, and [d]Molecular Gastroenterology Unit, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany

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Pancreatic ductal carcinoma · Microarray · Gene expression profiling · Cancer, pancreas

Abstract
Pancreatic ductal adenocarcinoma (PDAC) has an extremely poor prognosis. To improve the prognosis, novel molecular markers and targets for earlier diagnosis and adjuvant and/or neoadjuvant treatment are needed. Recent advances in human genome research and high-throughput molecular technologies make it possible to cope with the molecular complexity of malignant tumors. With DNA array technology, mRNA expression levels of thousand of genes can be measured simultaneously in a single assay. As several studies using microarrays in PDAC have already been published, this review attempts to compare the published data and therefore to validate the results. In addition, the applied techniques are discussed in the context of pancreatic malignancies.

Regine Brandt and Robert Grützmann contributed equally to this study.

Introduction

Pancreatic cancer is the fourth leading cause of cancer death in the United States [1] and the sixth leading cause of cancer deaths in Germany [2]. The World Health Organization estimates 112,000 cases worldwide for the year 2000. It is a common disease with a very poor prognosis. The frequent diagnosis at late stages of the disease limits the role of surgery as a curative modality. Despite advanced technologies in therapy and diagnostics, the 5-year survival rate remains less than 5% and generally the survival time after diagnosis is 3–6 months. About 95% of pancreatic cancers are ductal adenocarcinomas (PDACs), 5% are islet cell tumors. Development of pancreatic cancer is a multi-stage process resulting from the accumulation of genetic changes in somatic cells. Activating mutations of the k-ras oncogene are found in virtually all cases of pancreatic cancer and are early events in tumorigenesis [3–6]. Mutations in tumor suppressors, such as p16INK4a [7–9], p53 [8, 10–12] and DPC4/SMAD4 [13], appear to occur as a second step in advanced tumorigenesis. The identification of alterations in gene expression that occur during tumorigenesis may provide information for the early diagnosis of pancreatic cancer. Gene expression can be analyzed by various methods: differential dis-

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Jörg D. Hoheisel, PhD
Division of Functional Genome Analysis, Deutsches Krebsforschungszentrum
Im Neuenheimer Feld 580
DE–69120 Heidelberg (Germany)
Tel. +49 6221 424680, Fax +49 6221 424682, E-Mail j.hoheisel@dkfz.de

play RT-PCR; representational difference analysis; sequencing expressed sequence tags (ESTs) or short fragment tags produced during a process called sequential analysis of gene expression (SAGE), and finally DNA-microarray technology. All these methods were developed for comparative studies allowing a systematic screening for molecular differences at the level of mRNA expression in different cells or tissues.

Differential display is based on an amplification of messenger RNA 3′-termini using an anchored oligo-dT primer at one end and a short primer with an arbitrary sequence at the other end. The amplified cDNAs, labelled with a radioisotope, are then separated on a denaturing polyacrylamide gel and visualized by autoradiography or phosphorimaging. Side-by-side comparison of mRNA species from two or more related samples allows an identification of both up- and downregulation of the genes of interest [14].

Representational differential analysis [15] and related procedures take advantage of the competition between identical sequences for complementary DNA. In combination with a selective PCR amplification step, this allows a subtractive and kinetic enrichment of genes that are differentially transcribed in two samples. Such studies identified many genes that are over- or underexpressed in pancreatic cancer tissue [16, 17]. The procedure has the advantage of selecting with all transcripts; it lacks the ability of (easy) quantification, however.

The availability of large EST and SAGE-tag libraries make comparative EST or SAGE-tag counting possible. Libraries of identical sources can be pooled and the occurrence of sequences that are specific for a particular gene is determined by sequencing. Also, large databases of this kind exist (e.g., http://www.ncbi.nlm.nih.gov/) and can be used for in silico analyses. Tag counting has been successfully applied to pancreatic cancer [18]. Both methods, however, lack the potential for high-throughput analyses on a routine basis.

Recent developments in human genome research and technical advances in molecular biology procedures made DNA microarray technology the method of choice for gene expression analyses. This technology is capable to cope with the molecular complexity of malignant tumors, since the mRNA expression levels of thousands of genes can be measured simultaneously in a single assay. By such means, it is now possible to distinguish tumor subclasses by gene expression profiles and to identify new diagnostic and prognostic biomarkers as well as potential therapeutic targets.

Background of Chip Technology

The power of DNA microarrays as experimental tools derives from the specificity and relatively strong affinity of duplex formation of complementary sequences. In 1965, Gillespie and Spiegelman [19] observed that single-stranded DNA binds strongly to nitrocellulose membranes in a way that prevents the strands from re-associating with each other, but permits hybridization to complementary RNA. Development of a blotting technique by Southern [20] proved to be a milestone in assaying nucleic acids. Clone filter arrays were introduced by Lerach and colleagues [21] permitting a reverse analysis format in transcript analysis. Rather than studying global preparations of nucleic acids sequentially with individual probes, they presented the unique DNA content of very many recombinant library clones in an ordered manner, to which global RNA samples could be hybridized. The resulting parallelism in data production was a crucial step toward genome-wide analyses, as indicated by early results from pancreatic cancer samples done by Gress et al. [22]. In situ synthesis of oligonucleotide arrays [23, 24] and the arraying of prefabricated fragments of nucleic acids on glass surfaces [25] made such arrays more readily available.

Today, several options exist on how to create microarrays. Frequently, PCR products are arrayed in-house using spotting robots [26–28]. Also, a broad assortment of commercially available arrays exists [29]. Currently one of the most frequently used systems for global transcriptional profiling analysis is that from Affymetrix. Their arrays are produced by photolithographically controlled in situ oligonucleotide synthesis. Up to one million probes could be synthesized on an array of 1.28 × 1.28 cm [30]. A big disadvantage of this system is the fact that the chip design cannot be modified easily. Especially for tailor-made arrays assaying only the genes relevant to the respective disease, global arrays represent an expensive overkill. However, newly developed systems, such as the Geniom device developed by febit [31] for example, allow a flexible design of the probe composition. Based on electronically controlled micromirrors, the time-consuming and expensive production of photolithographic masks is avoided. Therefore, microarrays of any probe content can be produced on site, nevertheless using the high resolution of photo-controlled synthesis; only a file of oligonucleotide sequences is required. Also, the chip can be subdivided into up to eight separate entities, thereby adapting the number of probe molecules to the actual need of the respective analysis.

Table 1. Published PDAC microarray analyses

Author	Type of array	Number of genes	Tissues, number				Micro-dissection
			T	N	CP	CL	
Friess et al. [44], 2001	Oligonucleotides	~5,600	8	8 (organ donors)	8	–	No
Han et al. [46], 2002	cDNA PCR products	~5,289		1 (commercial)	–	8	No
Iacobuzio-Donahue et al. [47], 2002	Oligonucleotides	~12,000	14	11	–	8	No
Tan et al. [48], 2003	cDNA PCR products	~12,800	6	6	–	–	No
Iacobuzio-Donahueet al. [49], 2003	cDNA PCR products	~30,000	17	5	–	14	No
Logsdon et al. [50], 2003	Oligonucleotides	~5,600	10	5	5	7	No
Grützmann et al. [51], 2003	Oligonucleotides	~3,000	7	3	–	5	Yes
Crnogorac-Jurcevic et al. [52], 2003	cDNA PCR products	~9,000	9	4	–	20	No
Friess et al. [53], 2003	Oligonucleotides	~5,600	8	8	8	–	No
Iacobuzio-Donahue et al. [54], 2003	Oligonucleotides	~33,000	26	25 (+25 normal duodenal mucosa)	7	13	No
Terris et al. [55], 2002	cDNA PCR products	~4,992	13[a]	4[b]	–	1	

T = Tumor tissues; N = normal tissues; CP = chronic pancreatitis; CL = cell lines.

[a] Intraductal papillary-mucinous tumor.

[b] A pool of normal pancreas was prepared from 2 donor tissues and 2 normal adjacent pancreatic parenchymas from ampullary tumors.

Beyond transcriptional profiling, there is a large variety of other analysis types that can be performed on microarrays. The detection of single nucleotide polymorphisms [32], comparative genomic hybridization [33] or studies on epigenetic variation [34] for example, could become as important for diagnosis and prognosis as the analysis on the transcript level.

Advances in Research on Pancreatic Cancer Using Microarray Technology

Gene expression profiling has been applied to a variety of tumors such as breast [35], colon [36–38], prostate [39], esophagus [40] and the stomach [41]. In pancreatic cancer, several analyses using methods other than microarrays were performed to elucidate gene expression changes [17, 18, 42–45]. In addition, 11 studies of pancreatic tumors by DNA microarray technology have been published to date [44, 46–55] (table 1). These studies identified large sets of new class-II cancer genes [56] revealing dysregulation at the level of transcription. However, as noticed earlier [57], the published results are not easily comparable, because of the different hybridization platforms and data analysis procedures that were applied. In addition, the samples were not picked from a defined cohort of patients. Nevertheless, this article aims at an initial cross-validation of the published data on genes that are differentially expressed in normal and neoplastic pancreatic cells in order to define new markers or potentially interesting target entities, which are urgently required for an improved diagnosis and treatment.

Microarray Technology: How to Find Differentially Expressed Genes

Performing microarray experiments entails a twofold challenge. First and foremost is the correct performance of the experimental part. Already the preparation of the RNA is not easily performed and most critical to all subsequent steps especially in pancreatic tissues. Any difference in how the tissue is being isolated will immediately translate into changes in the transcript level. Given the fact that some mRNA molecules have a half life of a few seconds only, it is very likely that artificial variations will be seen for some mRNA molecules, regardless of the method applied. It is critical and possible, however, to freeze, literally, the transcript status of a tissue as quickly as technically possible in order to extract meaningful data.

Also the second part, the identification of significantly differentially transcribed genes from the total information generated on microarrays, is not trivial. To date, there is no gold standard for performing these bio-informatic

Table 2. Useful databases and software tools

Name	URL	Description
ArrayExpress	http://www.ebi.ac.uk/arrayexpress/	Data collection and software source
GEO	http://www.ncbi.nlm.nih.gov/geo/	Data collection and software source
SMD	http://genome-www5.stanford.edu/	Data collection and software source
M-CHiPS	http://www.mchips.org	Data collection and software source
SAM	http://www-stat.stanford.edu/~tibs/SAM/	Data analysis software
Base	http://base.thep.lu.se/	Open source database software
GenePattern	http://www-genome.wi.mit.edu/cancer/software/software.html	Data analysis software
BioConductor	http://biosun1.harvard.edu/complab/dchip/	Software source

analyses, but some general rules are emerging. Normalization, for example, is mostly performed globally based on an average intensity level on a microarray. Only rarely is it based on a selection of genes which are considered to be not differentially expressed [58, 59]. The former process is mostly much more robust. Still, it may lead to false interpretation [60]. Also, the definition of a globally applicable threshold is impossible, since such a value depends on all experimental factors and therefore differs from experiment to experiment. Only statistical analyses, such as the 'significance analysis of microarrays' package [61], permit a sensible analysis. Minimal standards on how to perform transcriptional profiling analyses on microarrays were discussed and defined by the MGED consortium (www.mged.org) [62]. Next to commercial software, several program packages exist that are freely available to academic users (table 2).

Gene Validation

If performed according to strict statistical procedures, the measurement of differential gene expression with microarrays can be a very reliable method. In a global setting, cross-validation of the results with other array experiments enables an evaluation of data quality, simply by increasing the number of experiments that are taken into account. The feasibility of this approach was demonstrated by Rhodes et al. [63] and is actually the aim of this review with respect to pancreatic cancer. For individual transcripts, quantitative PCR [64] is an even more precise instrument for the measurement of differential RNA levels but lacks power with respect to throughput. Last, it is well known that variations in RNA level do not necessarily correlate with variations in protein level. Therefore, analysis of the actual protein expression may eventually supersede transcript profiling, at least for target identification.

Transcript Variations in Pancreatic Tumors

The results from several groups show that gene expression profiling of pancreatic tumors using high-density arrays yields meaningful data, if performed appropriately. In total, 978 genes were found to be differentially transcribed in pancreatic tumors. Table 3 combines the results of the currently available publications listed in table 1. Only genes that were found in at least 2 studies are listed, producing a catalogue of 148 genes. There are several potential reasons for this low concordance between the studies. First, the type, histology and number of samples used (i.e., established cell lines or human pancreatic cancer cells) differed widely. Some studies included tumors other than PDAC, like neuroendocrine tumors or tumors of the papilla of Vater. This degree of variation is most likely true also for the samples representing normal tissues (commercially available RNA, normal tissue from resected pancreatic tumors or donor organs). Second, microdissection was applied by one group [51] while most studies were performed on whole tissue samples or cell lines. In cell lines, in vitro conditions may induce changes in gene expression that are not present in vivo. Pancreatic tumor specimens contain different cell types, including ductal, acinar, islet, inflammatory and nerve cells as well as fibrocytic elements. When whole tissues are used, the expression profiles represent both the variations in tumor and the adjacent non-neoplastic tissue. Third, different arrays and array technologies may lead to different gene expression results. As Kuo et al. [65] demonstrated, there can be poor correlation when the same samples are analyzed with two technologies (e.g., cDNA versus oligonucleotide microarrays). Fourth, statistical analysis and data mining procedures differed between the studies. Last, quite a few of the variations could well be due to individual reactions of the patients rather than being directly cancer-related.

Table 3. Genes shown to be differentially transcribed in pancreatic tumors using microarray technology

UG_ID	a	b	c	d	e	f	g	h	i	j	Counts		Acc	Name	Symbol
											T	N			
Hs.2962	T	T	T	T	T				T	T	7	0	NM_005980	S100 calcium-binding protein P	S100P
Hs.204238	T	T			T	T				T	5	0	NM_005564	Lipocalin 2 (oncogene 24p3)	LCN2
Hs.287558		T	T		T	T		T		N	5	1	NM_000700	Annexin A1	ANXA1
Hs.54451	T	T	T	T	T						5	0	NM_005562	Laminin-γ2	LAMC2
Hs.154036		T	T	T				T			4	0	NM_003311	Pleckstrin homology-like domain, family A, member 2	PHLDA2
Hs.179657	T			T	T	T					4	0	NM_002659	Plasminogen activator, urokinase receptor	PLAUR
Hs.194691	T	T			T			T			4	0	NM_003979	Retinoic acid induced 3	RAI3
Hs.251754	T		T		T			T			4	0	NM_003064	Secretory leukocyte protease inhibitor (anti-leukoproteinase)	SLPI
Hs.408864			T	T	T					T	4	0	NM_000574	Decay-accelerating factor for complement[1]	DAF
Hs.417004			T	T	T	T					4	0	NM_005620	S100 calcium-binding protein A11 (calgizzarin)	S100A11
Hs.82237	T	T		T					T		4	0	NM_012101	Tripartite motif-containing 29	TRIM29
Hs.82422	T	T	T		T						4	0	NM_001747	Capping protein (actin filament), gelsolin-like	CAPG
Hs.103707	T							T		T	3	0	XM_039877	Mucin-5, subtypes A and C, tracheobronchial/gastric	MUC5AC
Hs.111554	T	T			T						3	0	NM_005737	ADP-ribosylation factor-like 7	ARL7
Hs.1355			T		T					T	3	0	NM_001910	Cathepsin E	CTSE
Hs.139851	T	T			T						3	0	BC005256	Caveolin 2	CAV2
Hs.155419	T		T	T							3	0	NM_001197	BCL2-interacting killer (apoptosis-inducing)	BIK
Hs.156346	T	T	T								3	0	NM_001067	Topoisomerase (DNA) II α, 170 kDa	TOP2A
Hs.169902				T	T				T		3	0	NM_006516	Solute carrier family 2 (facilitated glucose transporter), member 1	SLC2A1
Hs.184510	T		T	T							3	0	NM_006142	Stratifin	SFN
Hs.220529	T			T	T						3	0	NM_004363	Carcinoembryonic antigen-related cell adhesion molecule 5	CEACAM5
Hs.2442			T		T			T			3	0	NM_003816	Disintegrin and metalloproteinase domain 9 (meltrin-γ)	ADAM9
Hs.2785	T			T	T						3	0	NM_000422	Keratin 17	KRT17
Hs.292738			T			T	T				3	0	NM_001175	Rho GDP dissociation inhibitor (GDI) β	ARHGDIB
Hs.2979	T		T							T	3	0	NM_005423	Trefoil factor 2 (spasmolytic protein 1)	TFF2
Hs.301350			T	T	T						3	0	NM_021910	FXYD domain containing ion transport regulator 3	FXYD3
Hs.309517			T	T	T						3	0	NM_002276	Keratin 19	KRT19
Hs.323733	T	T	T								3	0	NM_004004	Gap junction protein, β2, 26 kDa (connexin 26)	GJB2
Hs.348553	T				T			T			3	0	AK027130	Immortalization-upregulated protein	IMUP
Hs.362731		T			T	T					3	0	NM_013451	fer-1-like 3, myoferlin *(C. elegans)*	FER1L3
Hs.376874			T	T						T	3	0	NM_002245	Potassium channel, subfamily K, member 1	KCNK1
Hs.418138							T		T	T	3	0	NM_002026	Fibronectin 1	FN1
Hs.423190								T	T	T	3	0	NM_001311	Cysteine-rich protein 1 (intestinal)	CRIP1
Hs.450230			T	T	T						3	0	NM_000598	Insulin-like growth factor-binding protein 3	IGFBP3
Hs.624	T	T	T								3	0	NM_000584	Interleukin-8	IL8
Hs.83758			T			T		T			3	0	NM_001827	CDC28 protein kinase regulatory subunit 2	CKS2
Hs.85266			T	T					T		3	0	NM_000213	Integrin-β4	ITGB4
Hs.112341	T		T								2	0	NM_002638	Protease inhibitor 3, skin-derived (SKALP)	PI3
Hs.115166	T				T						2	0	NM_144777	Sciellin	SCEL
Hs.118400	T	T									2	0	NM_003088	Fascin homolog 1, actin-bundling protein *(Strongylocentrotus purpuratus)*	FSCN1
Hs.126222					T			T			2	0	NM_005717	Actin-related protein 2/3 complex, subunit5, 16 kDa	ARPC5
Hs.127799		T	T								2	0	NM_001165	Baculoviral IAP repeat-containing 3	BIRC3
Hs.128453			T							T	2	0	NM_001463	Frizzled-related protein	FRZB
Hs.136348		T							T		2	0	NM_006475	Osteoblast-specific factor-2 (fasciclin-I-like)	OSF-2
Hs.139389			T	T							2	0	NM_001323	Cystatin E/M	CST6
Hs.1600					T			T			2	0	NM_012073	Chaperonin-containing TCP1, subunit 5 (ε)	CCT5
Hs.172928									T	T	2	0	NM_000088	Collagen type I, α1	COL1A1
Hs.179729	T		T								2	0	NM_000493	Collagen type X, α1(Schmid metaphyseal chondrodysplasia)	COL10A1
Hs.180909			T		T						2	0	NM_002574	Peroxiredoxin 1	PRDX1
Hs.180919			T							T	2	0	NM_002166	Inhibitor of DNA-binding 2, dominant negative helix-loop-helix protein	ID2
Hs.191842			T	T						N	2	1	NM_001793	Cadherin 3, type 1, P-cadherin (placental)	CDH3
Hs.198281				T					T	N	2	1	NM_182470	Pyruvate kinase, muscle	PKM2
Hs.211573			T	T							2	0	NM_005529	Heparan sulfate proteoglycan 2 (perlecan)	HSPG2

➤

Table 3 (continued)

UG_ID	a	b	c	d	e	f	g	h	i	j	Counts		Acc	Name	Symbol
											T	N			
Hs.21486			T	T							2	0	NM_007315	Signal transducer and activator of transcription 1, 91 kDa	STAT1
Hs.21858						T	T				2	0	NM_006216	Serine (or cysteine) proteinase inhibitor, clade E[2]	SERPINE2
Hs.223025			T				T				2	0	NM_006868	RAB31, member RAS oncogene family	RAB31
Hs.226391					T				T		2	0	NM_006408	Anterior gradient-2 homolog *(Xenopus laevis)*	AGR2
Hs.227806	T	T									2	0	NM_170692	RAS protein activator-like 2	RASAL2
Hs.22941	T	T									2	0	NM_020792	KIAA1363 protein	KIAA1363
Hs.232115		T					T				2	0	NM_000089	Collagen type-I, α2	COL1A2
Hs.235782	T	T									2	0	NM_016354	Solute carrier organic anion transporter family, member 4A1	SLCO4A1
Hs.23881	T			T							2	0	NM_005556	Keratin 7	KRT7
Hs.241579	T	T									2	0	NM_001235	Serine (or cysteine) proteinase inhibitor, clade H[3]	SERPINH1
Hs.2499		T		T							2	0	NM_002741	Protein kinase C-like 1	PRKCL1
Hs.250641			T		T						2	0	NM_003290	Tropomyosin 4	TPM4
Hs.250822						T		T			2	0	NM_003600	Serine/threonine kinase 6	STK6
Hs.25338		T			T						2	0	NM_007173	Protease, serine, 23	SPUVE
Hs.265829				T	T					N	2	1	NM_002204	Integrin-α3 (antigen CD49C, α3 subunit of VLA-3 receptor)	ITGA3
Hs.268571		T	T								2	0	NM_001645	Apolipoprotein C-I	APOC1
Hs.272822	T				T						2	0	NM_003707	RuvB-like 1 *(E. coli)*	RUVBL1
Hs.275243				T					T		2	0	NM_014624	S100 calcium-binding protein A6 (calcyclin)	S100A6
Hs.278613				T				T			2	0	NM_005532	Interferon-α-inducible protein 27	IFI27
Hs.278896			T	T							2	0	NM_019075	UDP glycosyltransferase-1 family, polypeptide A10	UGT1A10
Hs.279651	T		T								2	0	NM_006533	Melanoma inhibitory activity	MIA
Hs.283565	T					T					2	0	NM_005438	FOS-like antigen 1	FOSL1
Hs.334562	T				T						2	0	NM_001786	Cell division cycle 2, G1 to S and G2 to M	CDC2
Hs.348935			N				T		T		2	1	NM_020070	Immunoglobulin-λ-like polypeptide 1	IGLL1
Hs.352018			T	T							2	0	NM_000593	Transporter 1, ATP-binding cassette, subfamily B (MDR/TAP)	TAP1
Hs.3628	T	T									2	0	NM_145686	Mitogen-activated protein kinase kinase kinase kinase 4	MAP4K4
Hs.387579			T		T						2	0	NM_001769	CD9 antigen (p24)	CD9
Hs.387725				T	T						2	0	NM_002203	Integrin-α2 (CD49B, α2 subunit of VLA-2 receptor)	ITGA2
Hs.38972	T				T						2	0	NM_005727	Tetraspan 1	TSPAN-1
Hs.406475							T		T		2	0	NM_002345	Lumican	LUM
Hs.406515			T	T							2	0	NM_000903	NAD(P)H dehydrogenase, quinone 1	NQO1
Hs.411701					T					T	2	0	NM_194327	Lectin, galactoside-binding, soluble, 3 (galectin 3)	LGALS3
Hs.411958			T		T						2	0	NM_018950	Major histocompatibility complex, class I, F	HLA-F
Hs.419776		T			T						2	0	NM_139207	Nucleosome assembly protein-1-like 1	NAP1L1
Hs.434488		T					T				2	0	NM_004385	Chondroitin sulfate proteoglycan 2 (versican)	CSPG2
Hs.436718				T		T					2	0	NM_002483	Carcinoembryonic antigen-related cell adhesion molecule 6[4]	CEACAM6
Hs.436983			T	T							2	0	NM_000228	Laminin-β3	LAMB3
Hs.445226			T	T							2	0	NM_004240	Thyroid hormone receptor interactor 10	TRIP10
Hs.512682			T	T							2	0	NM_001712	Carcinoembryonic antigen-related cell adhesion molecule 1[5]	CEACAM1
Hs.512708		T			T						2	0	NM_004613	Transglutaminase 2[6]	TGM2
Hs.512711					T					T	2	0	NM_000365	Triosephosphate isomerase 1	TPI1
Hs.55279				T					T		2	0	NM_002639	Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5	SERPINB5
Hs.56319			T	T							2	0	NM_001899	Cystatin S	CST4
Hs.727	T			T							2	0	NM_002192	Inhibin-βA (activin A, activin AB α-polypeptide)	INHBA
Hs.72925			T	T							2	0	NM_003475	Chromosome-11 open reading frame 13	C11orf13
Hs.75318				T	T						2	0	NM_006000	Tubulin-α1 (testis specific)	TUBA1
Hs.75360			T							T	2	0	NM_001873	Carboxypeptidase E	CPE
Hs.77274	T								T		2	0	NM_002658	Plasminogen activator, urokinase	PLAU
Hs.77348			T		T						2	0	NM_000860	Hydroxyprostaglandin dehydrogenase 15 (NAD)	HPGD
Hs.77515			T	T							2	0	NM_002224	Inositol 1,4,5-triphosphate receptor, type 3	ITPR3

Table 3 (continued)

UG_ID	a	b	c	d	e	f	g	h	i	j	Counts		Acc	Name	Symbol
											T	N			
Hs.79033			T							T	2	0	NM_012413	Glutaminyl-peptide cyclotransferase (glutaminyl cyclase)	QPCT
Hs.79440	T	T									2	0	NM_006547	IGF-II mRNA-binding protein 3	IMP-3
Hs.82101	T	T									2	0	NM_007350	Pleckstrin homology-like domain, family A, member 1	PHLDA1
Hs.82109			T		T						2	0	NM_002997	Syndecan 1	SDC1
Hs.82961									T	T	2	0	NM_003226	Trefoil factor 3 (intestinal)	TFF3
Hs.83450			T	T						N	2	1	NM_198129	Laminin-α3	LAMA3
Hs.83753					T	T					2	0	NM_003091	Small nuclear ribonucleoprotein polypeptides B and B1	SNRPB
Hs.85226			T		T						2	0	NM_000235	Lipase A, lysosomal acid, cholesterol esterase (Wolman disease)	LIPA
Hs.86947			T	T							2	0	NM_001109	Disintegrin and metalloproteinase domain 8	ADAM8
Hs.87268			T	T						N	2	1	NM_001630	Annexin A8	ANXA8
Hs.89434	T	T									2	0	NM_080881	Drebrin 1	DBN1
Hs.98428	T				T						2	0	NM_156036	Homeo box B6	HOXB6
Hs.123107			N					N	N		0	3	NM_002257	Kallikrein 1, renal/pancreas/salivary	KLK1
Hs.199695			N			N			N		0	3	NM_014573	Hypothetical protein MAC30	MAC30
Hs.26126			N			N			N		0	3	NM_182848	Claudin 10	CLDN10
Hs.375108						N	N	N			0	3	NM_013230	CD24 antigen (small cell lung carcinoma cluster 4 antigen)	CD24
Hs.409223			N			N			N		0	3	NM_006280	Signal sequence receptor, δ (translocon-associated protein δ)	SSR4
Hs.423			N					N	N		0	3	NM_138938	Pancreatitis-associated protein	PAP
Hs.75335			N				N		N		0	3	NM_001482	Glycine amidinotransferase (*L*-arginine:glycine amidinotransferase)	GATM
Hs.992			N					N	N		0	3	NM_000928	Phospholipase A2, group IB (pancreas)	PLA2G1B
Hs.25647						N		N		T	1	2	NM_005252	v-fos FBJ murine osteosarcoma viral oncogene homolog	FOS
Hs.107			N						N		0	2	NM_004467	Fibrinogen-like 1	FGL1
Hs.1239			N						N		0	2	NM_001150	Alanyl (membrane) aminopeptidase[7]	ANPEP
Hs.1340			N					N			0	2	NM_001832	Colipase, pancreatic	CLPS
Hs.169234			N						N		0	2	NM_015849	Pancreatic elastase IIB	ELA2B
Hs.169900			N						N		0	2	NM_003819	Poly(A)-binding protein, cytoplasmic 4 (inducible form)	PABPC4
Hs.181300			N						N		0	2	NM_005065	Sel-1 suppressor of lin-12-like *(C. elegans)*	SEL1L
Hs.2879			N					N			0	2	NM_001868	Carboxypeptidase A1 (pancreatic)	CPA1
Hs.388004			N				N				0	2	NM_000687	S-adenosylhomocysteine hydrolase	AHCY
Hs.407856			N					N			0	2	NM_003122	Serine protease inhibitor, Kazal type 1	SPINK1
Hs.410578			N				N				0	2	NM_000918	Procollagen-proline, 2-oxoglutarate 4-dioxygenase[8]	P4HB
Hs.422542			N					N			0	2	NM_004132	Hyaluronan-binding protein 2	HABP2
Hs.433391			N					N			0	2	NM_005950	Metallothionein 1G	MT1G
Hs.433750			N							N	0	2	NM_182917	Eukaryotic translation initiation factor 4γ, 1	EIF4G1
Hs.433797			N						N		0	2	NM_002885	RAP1, GTPase-activating protein 1	RAP1GA1
Hs.435699			N						N		0	2	NM_002771	Protease, serine, 3 (mesotrypsin)	PRSS3
Hs.436042			N					N			0	2	NM_000609	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	CXCL12
Hs.437638			N						N		0	2	NM_005080	X-box binding protein 1	XBP1
Hs.444159								N	N		0	2	NM_003627	Solute carrier family 43, member 1	SLC43A1
Hs.74502			N					N			0	2	NM_001906	Chymotrypsinogen B1	CTRB1
Hs.75462							N		N		0	2	NM_006763	BTG family, member 2	BTG2
Hs.79361			N						N		0	2	NM_002774	Kallikrein 6 (neurosin, zyme)	KLK6
Hs.79428			N						N		0	2	NM_004052	BCL2/adenovirus E1B 19 kDa interacting protein 3	BNIP3
Hs.80206			N							N	0	2	NM_000402	Glucose-6-phosphate dehydrogenase	G6PD
Hs.81131			N						N		0	2	NM_000156	Guanidinoacetate N-methyltransferase	GAMT

1 CD55, Cromer blood group system.
2 Nexin, plasminogen activator inhibitor type 1, member 2.
3 Heat shock protein 47, member 1, collagen binding protein 1.
4 Non-specific cross-reacting antigen.
5 Biliary glycoprotein.
6 C polypeptide, protein-glutamine-γ-glutamyltransferase.
7 Aminopeptidase N, aminopeptidase M, microsomal aminopeptidase, CD13, p150.
8 (Proline 4-hydroxylase), β-polypeptide (protein disulfide isomerase; thyroid hormone-binding protein p55).

a = Iacobuzio-Donahue CR2003 [54]; b = Iacobuzio-Donahue AJP2002 [47]; c = Friess CMLS2003 [53]; d = Logsdon CR2003 [50]; e = Iacobuzio-Donahue AJP2003 [49]; f = Han CR2002 [46]; g = Tan WJG2003 [48]; h = Grützmann VA2003 [51]; i = Crnogorac-Jurcevic JP2003 [52]; j = Terris AJP2002 [55]; T = overexpressed in tumor tissue; N = overexpressed in normal tissue.

The majority of the 148 genes (n = 115) were found to be upregulated in pancreatic tumors. One gene, S100 calcium-binding protein P, was upregulated in 7 of the 10 studies. Three genes, among them annexin-A1, lipocalin-2 and laminin-γ2, were upregulated in 5 studies. Eight genes, including gelsolin-like protein, S100 calcium-binding protein A11, plasminogen activator, urokinase receptor and retinoic acid induced 3, were overexpressed in 4 studies. Three of 10 was scored by 25 genes, among them ADAM9. Its expression was very recently found to be an independent marker of shortened survival after curative resection of PDAC [66]. The majority of genes (in total 78) were upregulated in 2 studies. Among the genes that were overexpressed in PDAC, 9 genes including keratin-7 [67], stratifin [68] and CD55 [69] had been previously implicated in PDAC. These genes, which have also been identified by other profiling methods, confirm the validity of microarray-based expression profiling. Moreover, 14 of the upregulated genes were known from microarray analyses on other cancers. This includes versican (overexpressed in malignant melanomas) [70], insulin-like growth factor-binding protein-3 (overexpressed in non-small cell lung cancer and breast cancer) [71, 72] and carcinoembryonic antigen-related cell adhesion molecule-6 (CEACAM6; overexpressed in colorectal carcinomas) [73]. These genes might play an important role in PDAC, too. The remaining 13 genes listed in table 3 have not before been implicated to be involved in carcinogenesis.

Only 33 genes were found to be downregulated in pancreatic tumors in 2 or 3 of the 10 publications, respectively, among them CD24 antigen and V-fos FBJ murine osteosarcoma viral oncogene homolog. These two genes had not been described to be of relevance in PDAC before. Interestingly, however, CD24 was found to be an independent prognostic marker in non-small cell lung cancer patients [74]. Several genes, among them nucleosome assembly protein-1-like 1 (NAP1L1), showed discrepant results. In two studies, NAP1L1 was described as upregulated [47, 50], whereas a downregulated status was detected with microdissected material [51].

Microarray Studies for Answering Clinical Questions

Microarray technology has been proven to be feasible in pancreatic tumors. Many novel candidate genes emerged from the initial studies. On the other hand, so far the expression profiles have not been proven to correlate with individual cancer stages, grading and TMN classification. Also, molecular differences between long- and short-time survivors have not been detected. Most probably, however, this is mainly due to the small number of samples in the studies mentioned above. New investigations are under way in several laboratories, including our own, and will eventually provide the information necessary. Already, the discrimination power of such analyses was found to be sufficient to define new tumor entities on the basis of their transcript profile [75].

The possibility of individualized medicine, the molecular correlation between responders and non-responders to adjuvant or neoadjuvant therapy, for example, is one of the aims in this line of research. This, however, requires carefully designed prospective profiling studies on hundreds of patients, possibly focusing the analysis on microdissected cells from normal and matched cancerous pancreatic tissues. Moreover, the approach only analyzes differences in the expression level of the genes, but not the activity of proteins. Therefore, interesting candidate genes have to be validated on the level of protein activity.

Conclusions and Perspectives

Due to the human genome project, sequence information of the entire human genome is available. In the form of DNA microarrays, it can be utilized for global analyses of gene expression. As opposed to earlier approaches, no well-defined working hypothesis is required in this methodology. It is a matter of comparing the transcript levels between different tissues, or between healthy and diseased tissue of the same kind. This approach exhibits both advantages and weak points. A major advantage is the fact that the analysis of gene expression is a totally unprejudiced process. No desires, assumptions or theories of the experimenter are incorporated in the investigations. Analysis and initial interpretation should be based purely on statistical means. Also, no pre-selection of genes is taking place. One of the major drawbacks is the fact that frequently the interpretation of the data is not immediately feasible. Further, for the lack of a hypothesis, a reasonably large amount of information has to be attained before a statistically significant conclusion can be phrased.

In conclusion, microarray-based transcript analyses have broadened our understanding of tumor biology. These data will influence diagnostic and therapeutic strategies for the treatment PDAC and other tumors. With the great progress made in the handling and analysis of microarrays, it can be assumed that more insights will

soon be gained into the fundamental changes occurring within a cancer cell. In future, and really not too far ahead, microarray technology will be broadly introduced into clinical practice. The discovery of novel therapeutically useful genes and diagnostic markers will fuel medical progress and lead eventually to novel drugs and diagnostic strategies.

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