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Review

Gene Expression and Proteomic Analysis of Pancreatic Cancer: a Recent Update

RU CHEN[1], SHENG PAN[2], DAVID A. CRISPIN[1] and TERESA A. BRENTNALL[1]

[1]GI Division / Department of Medicine, University of Washington, Seattle, WA 98195;
[2]Institute for Systems Biology, Seattle, WA 98103, U.S.A.

Abstract. *Pancreatic cancer is a lethal disease for which little progress in early diagnosis or treatment has been made for many decades. Better biomarkers are urgently needed for early detection while the cancer is potentially curable. Recently, expression profiling, including gene expression profiling and proteomic profiling, have demonstrated new opportunities to investigate crucial events underlying pancreatic tumorigenesis and to exploit this knowledge for early detection and better intervention. This review will discuss and compare recently published data on this topic.*

With the completion of the human genome sequencing project and the continued progress in technologies and bioinformatics tools, global analysis of gene and protein expressions in cells and tissues is becoming feasible. Differential expression profiling in cancer compared to normal or non-cancerous tissues allows for the parallel analysis of the genes and proteins that are expressed under such disease settings, leading to discovery of dysregulated genes/proteins that are biologically significant or responsible for the disease. Such efforts can potentially lead to the discovery of important biomarker candidates for diagnosis, prognosis and therapy. Pancreatic cancer is the fourth leading cause of cancer death in the United States (1), with one of the worst survival rates of all cancer types. While there has been considerable improvement in the genetic and proteomic understanding of the top three cancers in the US (breast, lung and colon), the fourth most common cause of cancer death (pancreatic) has not been adequately studied. Pancreatic cancer is diagnosed in 30,000 patients in the US every year; only 200 of these patients will still be alive one year later. Therefore, the study of genetic and protein events that lead to neoplastic progression in the pancreas is important from both scientific and clinical stand-points.

In the past ten years, many genetic alterations in pancreatic cancer have been elucidated (2, 3). For example, K-ras point mutations occur in over 90% of pancreatic cancers (2). Tumor suppressor genes, such as *p53, p16* and *DPC4,* are frequently inactivated (3), while epidermal growth factor receptor HER-2/neu becomes activated (4-6). However, the significant progress in the identification and characterization of cancer-related gene abnormalities has not been translated into substantial clinical improvement in the diagnosis or treatment of the disease. Efforts to use these genetic mutations as surrogate markers of disease have been unsuccessful due to their low sensitivity or specificity. Therapies directed to matrix metalloproteinase inhibitors (7-9), farnesyl transferase inhibitors (10) or epidermal growth factor receptor inhibitors (11) initially generated excitement that has waned due to the lack of substantive response in phase III clinical studies. Moreover, efforts to exploit these genetic events as potential biomarkers were unsuccessful because of the lack of the high sensitivity and specificity required to screen an asymptomatic population. The only widely used tumor marker, CA19-9, is frequently elevated in pancreatic cancer, but may also be expressed in other malignancies. Moreover, its levels can also be elevated in such benign conditions as acute and chronic pancreatitis, hepatitis or biliary obstruction (12).

The recent advances in technologies in genomics and proteomics have provided new tools and platforms for simultaneous global profiling of a large number of genes or proteins in two or multiple systems that can be compared. In pancreatic cancer, the studies have focused on the comparative profiling of the cancerous and normal pancreas, and have identified large numbers of differentially-expressed genes/proteins. The identification of gene and protein

Correspondence to: Ru Chen, Ph.D., Department of Medicine, University of Washington, Seattle, WA 98195, U.S.A. Tel: (206) 221-4109, Fax: (206) 685-9478, e-mail: ruchen@u.washington.edu

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expression alterations in cancer could provide a basis for the future development of diagnostic biomarkers, or prognostics biomarkers and lay the groundwork for future clinical research. This review discusses recent progress in the effort to identify differentially-expressed gene/proteins in pancreatic cancer tissue/cells.

Sample Preparation

Pancreatic ductal adenocarcinoma consists of a solid mass, with 30% to 90% of the tumor cells interspersed with a fibroblastic stroma. It is difficult to decide what the starting material should be for the development of biomarkers for cancer. One can start with purified cancer cells or with whole cancer including the non-cancerous components. Cross-interaction between tumor cells and the surrounding stroma is facilitated through proteins that modify cancer and stromal growth (13, 14). Profiling purified cancer cells may be an excellent starting source for biomarkers: the use of enriched cancer cells could facilitate the discovery of biomarkers present in small amounts. However, such an approach may miss biomarkers of cancer derived from the abnormal stromal cells. More than ten of the expression profiling studies used whole pancreatic cancer tissue, and identified a large set of dysregulated genes (15-25).

Several approaches have been applied to isolate pure cancer cells from pancreatic cancer tissue, including laser capture microdissection (LCM), CELLection Epithelial Enrichment and primary pancreatic ductal culture (26). CELLection Epithelial Enrichment utilizes super-paramagnetic polymer beads coated with the monoclonal antibody BerEP4 (EpCAM Ab) to isolate human epithelial cells from the cellular slurry of whole pancreatic cancer and has been demonstrated to be suitable for protein and mRNA studies (26, 27). Primary pancreatic ductal culture is a short-term cell culture approach to increase the quantity of a sample and is a particularly useful solution when dealing with small sample size (26). The most commonly used and versatile technique for cancer cell separation is LCM. The studies have shown that the use of LCM to microdissect epithelial cells from cancer tissues can effectively provide enriched populations of target cells (28-31). In the first application of microdissection for sample preparation in studying the gene expression profiling of pancreatic ductal adenocarcinomas, several differentially-expressed genes, that had not been previously reported, were discovered (28), suggesting the benefit of using microdissected material.

Gene Expression Profiling

In recent years, gene expression profiling has identified differentially-expressed genes in pancreatic cancers for applications as biomarkers, therapeutic targets and to increase the understanding of tumor progression. There are many published studies describing the application of gene expression profiling technologies to the analysis of cells and tissue from pancreatic cancer (15-25, 28-37). Most of these studies focused on the characterization of gene expression profiles and identification of specific genes that could serve as molecular targets for diagnosis and/or therapy. For the purposes of this review, we analyzed the data from seventeen studies of gene expression in pancreatic cancer (Table I). These studies were selected from a search in Pubmed with the key words 'pancreatic cancer', 'expression profiling' and 'gene expression'. In an attempt to construct a database containing all the published gene expression data, we found it can be challenging to comprehensively compare the results across different studies. With variations in gene expression tools, sample types and analytical tools, not all of the results from these studies are readily comparable. While the data from each of the studies was very informative, many genes were found to be dysregulated in one study but not in the others. Table II summarizes 144 genes that were identified and cross-validated by two or more different studies.

Among these 144 genes, 102 genes were up-regulated. The most frequent genetic alteration was overexpression of *S100P* (noted in six studies). Another gene, *TRIM29*, was up-regulated in five studies. Seven genes were overexpressed in four studies: *FOSL1, S100A11, SFN, KRT7, FN1,PLAU* and *PSCA*. Deregulation of mesothelin (*MSLN*) was shown in four studies, however, it was up-regulated in two of the studies, and down-regulated in the other two studies. In general, however, the direction of deregulation (*e.g.* up-regulation *versus* down-regulation) in these studies was in agreement.

Down-regulation of gene expression in pancreatic cancer was less common than up-regulation; only 42 genes were shown to be down-regulated in at least two studies. Among them, three genes, *MUC5B, FCGBP* and *PAP,* were down-regulated in three studies. All other down-regulated genes were only reported in one or two studies.

Quantitative Proteomics

While gene expression profiling is a powerful platform for identifying differentially-expressed genes, the corresponding protein levels are less well known. It is evident that RNA levels do not necessarily correlate with protein levels (38, 39). With the recent progress in mass spectrometry-based technology, global proteomics profiling of complex biological samples is becoming possible.

The recent development in proteomics has stimulated considerable interest in applying the technologies for

Table I. *Gene expression analysis of pancreatic cancer.*

Year	Author	Type of array	Test samples	Reference samples
2004	Aguirre *et al.*	cDNA microarray	24 pancreatic adenocarcinoma cell lines and 13 primary tumor specimens	human cDNA microarrays containing 14,160 cDNA clones
2002	Crnogorac-Jurcevic *et al.*	cDNA arrays	3 pancreatic ductal adenocarcinomas as microdissected	microdissected normal from 3 pancreatic cancer cases
2003	Crnogorac-Jurcevic *et al.*	cDNA arrays	9 pancreatic carcinoma	3 normal pancreata
2003	Friess *et al.*	oligonucleotide microarrays	8 pancreatic carcinoma	8 normal pancreata
2005	Fukushima *et al.*	oligonucleotide microarrays	3 pancreatic cancer, LCM microdissection	3 normal pancreas, LCM microdissection
2003	Grutzmann *et al.*	oligonucleotide microarrays	7 pancreatic cancer, LCM microdissection, and 5	3 normal pancreas, LCM microdissection
2005	Gysin *et al.*	cDNA microarrays	4 pancreatic cancer cell lines	pooled reference cell lines
2002	Han *et al.*	cDNA microarrays	9 pancreatic cancer cell lines	2 normal pancreas
2004	Holzmann *et al.*	Matrix-CGH	13 pancreatic cancer cell lines and 6 pancreatic tumor	reference human genomic DNA
2002	Iacobuzio-Donahue *et al.*	oligonucleotide microarrays	14 pancreatic adenocarcinoma, 8 pancreatic cancer cell lines	11 normal pancreas
2003	Iacobuzio-Donahue *et al.*	oligonucleotide microarrays	26 pancreatic adenocarcinoma tissues, 13 cell lines	50 normal tissues
2003	Logsdon *et al.*	oligonucleotide microarrays	10 pancreatic adenocarcinoma, 7 pancreatic cancer cell lines	5 normal pancreas
2004	Missiaglia *et al.*	cDNA arrays	19 pancreatic cancer cell lines	immortalized HPDE cell line
2004	Nakamura *et al.*	cDNA microarrays	10 pancreatic cancer, LCM microdissection	7 normal pancreas
2003	Tan *et al.*	cDNA microarrays	6 pancreatic carcinoma	adjacent normal tissues
2005	Prasad *et al.*	cDNA microarrays	microdissected early PanIN	microdissected normal duct epithelium
2005	Qian *et al.*	oligonucleotide microarrays	immortalized HPDE cell line/K-ras (G12V)	immortalized HPDE cell line

LCM, laser capture microdissection.

clinically-related research (40, 41). Substantial interest has been focused on applying proteomic methods for the discovery of new therapeutic targets, as well as the identification of new disease markers for diagnosis and early detection (41). A number of studies have been published employing proteomics to study the protein profile of pancreatic cancer (12, 42-46). Table III lists some of the recent publications specifically focused on studying the protein profile of tissues/cells from pancreatic cancer in comparison with normal samples.

Several of these studies were based on two-dimensional gel electrophoresis (2-DE). Microdissected malignant ductal epithelial cells from pancreatic cancer were studied using non-malignant cells as a control for protein profiling by 2-DE (44). Nine protein spots that were differentially-regulated were discovered, and 1 protein spot was identified as S100A6, which belongs to the S100 protein family. Several members of the S100 family have been reported to be overexpressed in pancreatic cancer both by mRNA and immunohistochemical analyses and, thus, may be important in pancreatic cancer. To overcome the technical difficulty due to limited microdissected material, DIGE (fluorescence difference gel electrophoresis) saturation labelling and 2-DE were applied to analyze microdissected cells from PanINs (pancreatic intraepithelial neoplasia) (46). The use of DIGE saturation labelling improved the sensitivity and enabled the successful 2-DE analysis of the samples. The study revealed 8 differentially-expressed proteins in microdissected PanIN regions, which included 3 actin filament-associated proteins and, thus, suggested a relevant role of the actin cytoskeleton during pancreatic tumor progression.

Whole pancreatic cancer tissue has also been used to study pancreatic adenocarcinoma using 2-DE in several studies (43, 45). In such a study (45), 40 differentially-expressed proteins were identified. A considerably higher number of proteins, compared to other 2-DE studies using microdissected samples, was identified in the study because it used whole cancer tissue, thus more sample was available for 2-DE analysis. Another 2-DE study on pancreatic adenocarcinoma revealed 111 differentially-expressed proteins related to pancreatic cancer (43).

Table II. *Genes differentially-expressed from at least two studies.*

Gene symbol	Locus	UP/DN	No. of studies	Reference	Gene symbol	Locus	UP/DN	No. of studies	Reference
S100P	4p16	UP	6	28, 23, 24, 21, 22, 31	*CFL1*	11q13	UP	2	31, 30
TRIM29	11q23	UP	5	28, 21, 18, 23, 31	*COL3A1*	2q31	UP	2	28, 31
FOSL1	11q13	UP	4	19, 20, 24, 22	*CCND1*	11q13		2	20, 22
S100A11	1q21	UP	4	19, 23, 22, 31	*DBN1*	5q35.3	UP	2	21, 22
SFN	1p36.11	UP	4	33, 31, 23, 22	*DAF*	1q32	UP	2	23, 24
KRT7	12q12	Up	4	21, 23, 22, 28	*DDAH1*	1p22	DN	2	31, 30
MSLN	16p13.3		4	31, 23, 22, 33	*SMAD4*	18q21.1		2	33, 18
FN1	2q34	UP	4	33, 3116, 21	*ELA1*	12q13	DN	2	31, 20
PLAU	10q24	UP	4	21, 31, 22, 28	*EVL*	14q32.32	DN	2	30, 31
PSCA	8q24.2	UP	4	21, 31, 22, 33	*EPHA4*	2q36.1	UP	2	31, 23
ACTB	7p15-p12		3	28, 22, 31	*FAP*	2q23	UP	2	21, 20
ANXA1	9q12-q21.2	UP	3	19, 30, 22	*FSCN1*	7p22	UP	2	21, 31
ARL7	2q37.2		3	21, 31, 22	*FOXM1*	12p13	UP	2	31, 20
FER1L3	10q24	UP	3	19, 21, 22	*LGALS1*	22q13.1	UP	2	22, 28
DDX21	10q21	UP	3	19, 21, 31	*GATM*	15q14	DN	2	28, 31
LCN2	9q34	UP	3	19, 21, 22	*GSTT1*	22q11.23		2	19, 15
PLAUR	19q13	UP	3	19, 23, 21	*GAPDH*	12p13	UP	2	28, 20
CDH3	16q22.1	UP	3	31, 23, 22	*HSPA1A*	23q13		2	31, 15
CEACAM6	19q13	UP	3	19, 23, 22	*HS3ST1*	4p16		2	31, 22
CAV2	7q31.1		3	21, 31, 22	*HMGA2*	12q15	UP	2	20, 31
KRT19	17q21.2	UP	3	33, 23, 22	*HRH4*	18q11.2	UP	2	15, 20
CLDN1	3q28-q29	UP	3	21, 19, 31	*HTATIP*	11q13	DN	2	31, 20
COL1A2	7q22.1	UP	3	28, 21, 31	*HABP2*	10q26.11	DN	2	31, 30
COL1A1	17q21.3	UP	3	28, 21, 31	*INHBA*	7p15-p13	UP	2	23, 22
C1S	12p13	UP	3	31, 20, 24	*IRS1*	2q36		2	20, 24
C2	6p21.3	UP	3	31, 15, 34	*IGFBP3*	7p13-p12	UP	2	23, 22
CST6	11q13	UP	3	23, 30, 24	*ITGB4*	17q11	UP	2	23, 28
FCGBP	19q13.1		3	31, 15, 24	*KRT17*	17q12	UP	2	23, 22
FXYD2	11q23		3	20, 31, 28	*KIAA1199*	15q	UP	2	21, 22
GJB2	13q11-q12	UP	3	15, 22, 21	*KIAA1363*	3q26	UP	2	21, 22
IFITM1	11p15.5	UP	3	21, 31, 24	*KLK1*	19q13.3		2	28, 30
IFI27	14q32	UP	3	31, 23, 30	*LGALS2*	22q12-q13	DN	2	31, 28
LAMA3	18q11	UP	3	23, 15, 20	*STMN1*	1p36.1-p35	UP	2	21, 31
LAMC2	1q25-q31	UP	3	21, 22, 23	*HLA-DRA*	6p21.3	UP	2	15, 30
MMP11	22q11.23	UP	3	31, 22, 28	*NNMT*	11q23.1	UP	2	21, 22
MUC5B	11p15		3	31, 22, 28	*NOTCH4*	6p21.3	UP	2	15, 28
PAP	2p12	DN	3	31, 22, 30	*NCOA3*	20q12	UP	2	31, 18
PHLDA1	12q15	UP	3	21, 20, 22	*NR4A1*	12q13	DN	2	31, 20
RAI3	12p13	UP	3	21, 31, 22	*NR4A2*	2q22-q23	DN	2	31, 24
SLP1	20q12	UP	3	28, 30, 22	*PNLIP*	10q26.1	DN	2	31, 20
THBS2	6q27	UP	3	21, 31, 22	*PITPNM1*	11q13		2	20, 31
TOP2A	17q21-q22	UP	3	21, 22, 33	*PKM2*	15q22	UP	2	28, 23
MMP7	11q21-q22	UP	3	21, 22, 28	*TSSC3*	11p15	UP	2	23, 21
ANXA4	2p13		2	31, 24	*PLEC1*	8q24	UP	2	21, 22
CD24	6q21	DN	2	19, 30	*KCNA5*	12p13	UP	2	31, 20
CYCS	7p15.2	UP	2	19, 31	*POU5F1*	6p21.31	DN		31, 15
DAP	5p15.2	DN	2	19, 30	*PRC1*	15q26.1	UP	2	31, 24
CAPG	2p11.2	UP	2	21, 22	*RAB2*	8q12.1	UP	2	31, 15
CLIC1	6p22.1	UP	2	15, 34	*RBMS1*	2q24.3	UP	2	31, 21
PRIM1	12q13	UP	2	19, 20	*RUNX1*	21q22.3	UP	2	22, 21
SSR4	Xq28	DN	2	19, 28	*S100A6*	1q21	UP	2	23, 28
TCEA1	3p21	UP	2	19, 31	SPP1	4q21-q25		2	21, 31

continued

Table II. *continued*

Gene symbol	Locus	UP/DN	No. of studies	Reference	Gene symbol	Locus	UP/DN	No. of studies	Reference
TPI1	12p13		2	20, 30	*SGK*	6q23	DN	2	31, 24
CPA1	7q32	DN	2	31, 30	*SMURF1*	7q21.1-q31.1	UP	2	15, 24
CLPS	6pter-p21.1	DN	2	31, 30	*SLC16A3*	17q25	UP	2	21, 22
TPM2	9p13.2-p13.1	UP	2	23, 15	*SLC2A1*	1p35-p31.3	UP	2	31, 23
ADAM8	10q26.3	UP	2	31, 23	*SLCO4A1*	20q13.33	UP	2	22, 15
FOS	14q24.3	DN	2	19, 30	*TERT*	5p15.33		2	15, 28
AK3	9p24.1	UP	2	31, 23	*TFF3*	21q22.3		2	28, 31
ADRM1	20q13.33	UP	2	15, 30	*TIMP1*	Xp11.3	UP	2	28, 21
AREG	4q13-q21	DN	2	31, 28	*ARNTL2*	12p12.2	UP	2	21, 22
ANXA13	8q24.13	DN	2	31, 20	*TTF2*	1p22	UP	2	15, 20
ANXA8	10q11	UP	2	23, 34	*TM4SF4*	3q25	DN	2	31, 24
ASPH	8q12.1	UP	2	21, 22	*TMEPA1*	20q13.31	UP	2	21, 22
ATP1B3	3q23	UP	2	31, 24	*TNF*	6p21.3	UP	2	18, 15
CABYR	18q11.2	UP	2	15, 20	*TACSTD2*	1p32-p31	UP	2	21, 22
CEACAM5	19q13	UP	2	23, 22	*VIM*	10p13		2	28, 30
CTSF	11q13	DN	2	31, 30	*MYBL2*	20q13.1	UP	2	31, 30
CD83	6p23	UP	2	31, 21	*MYC*	8q24.12-24	UP	2	18, 19
CDC20	1p34.1	UP	2	31, 30	*ZNF521*	18q11.2	UP	2	15, 20
CKS2	9q22	UP	2	31, 30	*CSPG2*	5q14.3	UP	2	31, 21

UP, up-regulated; DN, down-regulated.

Table III. *Proteomic analysis of pancreatic cancer.*

Year	Author	Analysis method	Test samples	Reference samples
2003	Cecconi *et al.*	2-DE/MS mass mapping	pancreatic adenocarcinoma cell line	pancreatic adenocarcinoma cell line
2003	Shekouh *et al.*	2-DE/MS mass mapping	malignant ductal epithelial cells	non-malignant ductal epithelial cells
2004	Lu *et al.*	2-DE/MS mass mapping	pancreatic adenocarcinoma	normal pancreas
2004	Shen *et al.*	2-DE/MS mass mapping	pancreatic adenocarcinoma	normal pancreas
2005	Chen *et al.*	Multi-dimentional LC/ICAT/MS/MS	pancreatic adenocarcinoma	normal pancreas
2005	Sitek B *et al.*	2-DE/MS/MS	microdissected PanIN cells	pancreatic carcinoma tissue

2-DE/MS, two-dimensional gel electrophoresis/mass spectrometry; LC, liquid chromatography; ICAT, isotope-coded affinity tag; PanIN, pancreatic intraepithelial neoplasia.

The recently developed isotope-coded affinity tag (ICAT) technology provides a more comprehensive approach for quantitative proteomic analysis (47). This methodology has demonstrated a significant improvement over gel-based methods in identifying proteins of low abundance (48). We recently applied ICAT technology to perform quantitative protein profiling of pancreatic cancer tissues compared to normal pancreas (12). The study identified 151 differentially-regulated proteins in cancer samples. Over half of these proteins are active in metabolism and cellular physiological processes. In addition, many of these differentially-regulated proteins are involved in signal transduction, cell growth and/or maintenance (12, 26). Moreover, many of the differentially-expressed proteins play a role in the communication system through which epithelial and tumor cells interact with the extracellular matrix. These protein-driven interactions orchestrate tumor growth, migration, angiogenesis, invasion, metastasis and immunological escape. The study revealed numerous proteins that are newly-discovered to be associated with pancreatic cancer, providing candidates for future early diagnosis biomarkers and targets for therapy. Several differentially-regulated proteins

Table IV. *Proteins differentially expressed from at least two studies.*

Gene symbol	Locus	UP/DN	No. of studies	Reference	Gene symbol	Locus	UP/DN	No. of studies	Reference
ANXA2	15q21-q22	UP	3	46, 12, 43	*FLNA*	Xq28	UP	2	43, 12
ANXA4	2p13		3	46, 45, 12	*FSCN1*	7p22	UP	2	43, 12
CPA1	7q32	DN	3	45, 43, 12	*GATM*	15q14	DN	2	43, 12
CPA2	7q32	DN	3	45, 12, 43	*GSN*	9q33-q34.1	UP	2	43, 12
CTRB1	16q23-q24.1	DN	3	45, 12, 43	*HSPA8*	11q24.1		2	45, 46
CTSD	11p15.5	UP	3	45, 12, 43	*IGKC*	2p12	UP	2	12, 43
ELA3A	1p36.12		3	45, 12, 43	*LGALS1*	22q13.1	UP	2	12, 45
GAPDH	12p13	UP	3	12, 46, 42	*LGALS3*	14q21-q22		2	46, 43
HSPA5	9q33-q34.1	DN	3	43, 12, 45	*LUM*	12q21.3		2	12, 43
PNLIPRP1	10q25.3		3	43, 12, 45	*PGK1*	Xq13	UP	2	46, 12
PPIA	19p13.2		3	46, 43, 45	*PNLIP*	10q26.1	DN	2	43, 12
PRSS1	7q34	DN	3	44, 43, 45	*PP*	10q11.1	UP	2	46, 43
ACTB	7p15-p12		2	46, 12	*PRDX1*	1p34.1		2	45, 46
ACTG1	17q25	UP	2	46, 12	*PRDX2*	19p13.2	DN	2	45, 46
ALB	4q11-q13		2	46, 12	*PRSS2*	7q34	DN	2	45, 43
AMY2A	1p21	DN	2	12, 45	*REG1A*	2p12	DN	2	12, 45
ANXA1	9q12-q21.2	UP	2	43, 12	*S100A9*	1q21		2	46, 43
CAPG	2p11.2	UP	2	46, 43	*TAGLN*	11q23.2		2	46, 43
CLPS	6pter-p21.1	DN	2	45, 12	*TGM2*	20q12	UP	2	12, 43
CPB1	3q24	DN	2	43, 12	*TPM2*	9p13.2	UP	2	45, 12
ELA3B	1p36.12	DN	2	45, 12	*TPM4*	19p13.1		2	45, 12
ENO1	1p36.3-p36.2		2	46, 43	*VIM*	10p13		2	46, 12

identified in this study were validated through Western blotting and immunohistochemical analysis on tissue arrays and might proved to be robust biomarker candidates (12).

Compared to gene expression profiling, protein profiling identified relatively fewer differentially-expressed proteins in pancreatic cancer. Forty-four proteins, that were abnormally expressed in the proteomics experiments, have been shown to be differentially-expressed in more than one protein profiling study (Table IV): 12 proteins were shown in three studies and 32 proteins were shown in two studies. The factors contributing to this low concordance between these protein profiling studies include the different proteomic profiling methods used, different histology for the samples, the use of microdissection and differences in the normal reference samples.

Genomic Approach *vs.* Proteomic Approach

There is a need to correlate changes in gene expression profiles with changes in proteomics profiles (49). Given the different regulation at the mRNA and protein levels, integration of gene expression profiles and protein profiling in cells and tissue will increase knowledge about the functions and regulatory pathways. For example, in one study, the comparison of gene expression patterns and the levels of 52 cancer-related proteins in 60 human cancer cell lines has led to an interesting observation in the integration of the protein and mRNA data: the levels of structural proteins were highly-correlated with the levels of their corresponding mRNAs, whereas the levels of non-structural proteins were poorly-correlated with their mRNAs (49, 50).

For pancreatic cancer, no systematic investigation to correlate the gene expression profile and the proteomics profile in the same sample has been reported to date. However, as a rudimentary analysis, we can correlate the published gene expression data and proteomics profiling data in pancreatic cancer. In the thousands of genes/proteins analyzed, only 66 genes/proteins have been shown to be dysregulated in pancreatic cancer by both techniques. The most frequently detected genes/proteins were: *ACTB, ANXA4, ANXA1, CPA1, SFN, KRT7* and *FN1,* which have been shown in five studies. Interestingly, the overexpression of S100 calcium-binding protein P (S100P), reported in six gene expression studies, has not been shown in any proteomic profiling study. As noted before, RNA levels do not necessarily correlate with protein levels (38, 39). In addition, technical differences, including the method used, in gene expression or proteomics, may also contribute to this difference.

Discussion

Gene expression profiling and proteomic profiling are envisioned as powerful tools for the identification of biomarkers for diagnosis, prognosis, therapy and predictive medicine, as well as for tumor classification. Most of the studies published to date have demonstrated the power of these techniques for the identification of differentially-expressed genes/proteins that could be used for future biomarker development. In some cancers, proof-of-principle studies have suggested the value of using expression profile approaches for tumor classification. For example, in lung cancer, studies have shown the potential of using gene expression profiles as classifiers to define subsets of lung cancer (51-53). Moreover, studies have shown that gene expression profiling can correlate with lung cancer prognosis (52). In pancreatic cancer, expression profiling approaches have proved powerful in identifying differentially-expressed genes/proteins that could potentially influence diagnosis and therapy. Further studies are needed to explore the potential of using expression profiling for cancer classification and prognosis prediction in pancreatic cancer.

With the maturity of gene expression techniques and the rapid development of proteomic techniques, it is now feasible to study and integrate global gene and protein expression profiles. In combination with other techniques, such as tissue arrays, ELISA and protein arrays, we can now obtain comprehensive information to study the disease mechanism and identify candidate biomarkers for pancreatic cancer. The differentially-regulated genes/proteins discovered in such studies will provide insights into a variety of cell functions and biological processes and broaden our understanding of pancreatic tumor biology. It is expected that expression technologies will continue to play an important role in clinical studies in the battle against cancer, towards the development of predictive, preventative and personalized medicine (54).

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