World J. Surg. 29, 297–305 (2005)
DOI: 10.1007/s00268-004-7843-0

WORLD Journal of SURGERY

Gene Overexpression in Pancreatic Adenocarcinoma: Diagnostic and Therapeutic Implications

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Published Online: February 10, 2005

Abstract. Pancreatic cancer is the deadliest of cancers, and effective diagnostic and therapeutic strategies are lacking. Global gene expression profiling holds promise for improved diagnosis and treatment. Knowledge of the location and timing of gene overexpression and the function of these genes, including their effects on signaling pathways, is important. These data may be used to develop histologic and serum biomarkers as well as to develop immunotherapeutic, molecular targeting, and gene therapy strategies. We have compiled a list of overexpressed genes in pancreatic cancer for which overexpression was confirmed by reverse transcriptase polymerase chain reaction, immunohistochemistry, and/or in situ hybridization following initial identification by global gene expression profiling. The techniques used in the determination of overexpression, problems encountered in global gene expression profiling, and the diagnostic and therapeutic implications of overexpression are discussed. The S100 gene family, mesothelin, prostate stem cell antigen, and 14-3-3 sigma, may have important clinical implications in pancreatic cancer diagnosis and treatment.

Pancreatic cancer is the fourth leading cause of cancer deaths in the United States. The mortality rate is the highest among cancer types, with an overall 5-year survival rate of less than 5% [1]. Because of its asymptomatic nature in early stages and a lack of sensitive and specific diagnostic tools, pancreatic cancer is usually detected after metastasis has occurred and when curative therapy is no longer possible. There are currently no biomarkers available for early detection of the disease, and even the most aggressive monitoring of high-risk patients is inadequate. Furthermore, pancreatic cancer is highly resistant to both chemotherapy and radiation therapy. The identification of overexpressed genes in pancreatic cancer may lead to the development of new biomarkers and screening techniques as well as new immunotherapeutic, molecular targeting, and gene therapy strategies.

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Techniques Used in the Identification of Gene Overexpression

The progression of carcinogenesis is thought to occur through the accumulation of multiple genetic alterations, and high-throughput gene expression technologies ("global gene expression profiling") have been recently used to simultaneously determine the expression patterns of thousands of genes.

These technologies are based either on serial, partial sequencing of cDNAs derived from sample RNA (as in serial analysis of gene expression [SAGE]), or on hybridization of labeled sample-derived cDNAs to specific probes immobilized on a grid (as in cDNA microarrays) [2, 3]. The SAGE technique has the advantage of being able to quantify gene expression without prior sequence information.

Recent global gene expression profiling studies of pancreatic cancer have employed SAGE, cDNA microarray, and oligonucleotide microarray technology to quantify gene expression in cancer cell lines as well as in human tissues. Several overexpressed genes have been identified by these techniques, and overexpression of many of these genes has been confirmed by individual studies of mRNA and/or protein expression. RNA studies have included Northern blots, in situ hybridization, and reverse transcriptase polymerase chain reaction (RT-PCR). Protein studies have included immunohistochemical labeling and Western blots. Gene expression studies which incorporate *both* RNA and protein analyses are ideal, as posttranslational regulation of gene expression frequently accounts for a disparity between mRNA abundance and protein expression.

Analysis of protein overexpression is particularly important for the development of biomarkers of cancer and for the identification of new therapeutic targets. Differential display proteomics, a biochemical technique that compares protein levels between tissue or fluid samples, allows for the simultaneous differential analysis of a large number of proteins and holds promise for improved diagnosis and therapy in pancreatic cancer [4].

Immunohistochemical staining and in situ hybridization are particularly useful as complements to global gene expression

profiling studies performed on bulk tumor, as they are able to localize the site of expression to a particular area of the tumor or to a particular cell type. Newly developed tissue microarray technology allows for large-scale immunohistochemical comparison of a multitude of tissue samples and has recently been used to verify differential protein expression between pancreatic cancer tissue samples, other tumor samples, and normal tissue [5].

Problems Encountered in Global Gene Expression Profiling

One problem with gene profiling performed by comparing pancreatic cancer tissue to normal pancreas is that the desmoplastic reaction of the tumor's stromal component contributes significantly to measured differences in overall gene expression. Interestingly, the desmoplastic response in chronic pancreatitis mimics that of pancreatic adenocarcinoma and accounts for fewer differentially expressed genes. A recent study by Logsdon et al. [6] incorporated a comparison between adenocarcinoma and chronic pancreatitis into its gene profiling strategy in order to more specifically identify genes differentially expressed by the neoplastic epithelium. The study's immunohistochemical localization of four of these genes, (*14-3-3 sigma/stratifin, beta 4 integrin, S100A6*, and *S100P*) to the neoplastic epithelium validated this approach.

Additional strategies to eliminate the influence of the desmoplastic response on differential gene expression measurements have included fine-needle aspiration of the tumor [7], laser capture microdissection [8], and comparison of pancreatic cancer cell lines (which lack a stromal component) to normal pancreas [9]. Laser capture microdissection, however, has proven to be time-consuming and labor-intensive, and the amount of genetic material obtained is minimal and usually requires amplification [7]. However, studies that profile cancer cell lines in vitro may not accurately reflect differential gene expression, as the cell lines lack in vivo influences on gene expression, including those of the surrounding stroma and the immune system [6].

Another problem facing gene expression profiling is that tumor gene expression is largely ductal, whereas the ductal cell contribution to gene expression in normal pancreas is diluted by the presence of islet and acinar cells. Therefore, genes appearing to be overexpressed in global gene profiling studies of tumor tissue may simply be attributable to the smaller ductal component of the normal sample rather than to neoplastic differential gene expression. To account for this problem, normal ductal cell lines have been used as a control in gene expression analysis, and differentially expressed genes found in studies involving normal tissue have been referenced in SAGE libraries of normal ductal cell lines, and excluded accordingly.

Finally, many of the genes and gene products identified as being differentially expressed in global gene expression profiling studies have several names or abbreviations and are not yet fully described in terms of location and function [10]. Further progress in these studies will be limited by the amount of information available for each individual gene.

Review of Differentially Expressed Genes

Global gene expression profiling studies, including SAGE, cDNA microarray, and oligonucleotide microarray analyses, were identified in the literature by a MEDLINE search. Genes for which overexpression was confirmed by Northern blot, in situ hybridization, RT-PCR, or immunohistochemistry following initial identification by global gene expression profiling are listed in Table 1. In general, the fact that these genes were chosen for confirmatory testing implies that they showed significant differential expression in global gene expression profiling, or that they have been implicated in carcinogenesis or are otherwise associated with cancer.

Each gene listed in Table 1 was then referenced in the Cancer Genome Anatomy Project (CGAP) Gene Finder and the SAGE Anatomic Viewer (http://cgap.nci.nih.gov/Genes/) in order to compare the number of tags identified in normal pancreatic DNA libraries with the number found in cancerous pancreatic DNA libraries. The widest discrepancy in data was chosen from among the three data options (*tissue only, cell lines only, tissue and cell lines)* and is shown in Table 2. Additional tissue types in which greater than 63 tags per 200,000 were observed in cancer are also included in Table 2.

Diagnostic Implications of Gene Overexpression

The identification of genes that are overexpressed in neoplastic pancreatic tissue versus non-neoplastic pancreatic tissue holds promise for the development of histologic diagnostic markers detectable in small biopsy or cytopathology samples. Furthermore, the identification of secretory or cell-surface gene products that are overexpressed in neoplastic pancreatic tissue versus other tumors or other normal tissues holds promise for the development of biomarkers detectable in the serum and/or pancreatic juice. It should be noted that the overall sensitivity and/or specificity of new diagnostic strategies and screening techniques may be optimized by incorporating a panel of markers that, individually, may lack sensitivity and/or specificity. Therefore, genes that are not exclusively overexpressed in neoplastic pancreatic tissue still warrant consideration as future diagnostic markers.

Identifying genes that are overexpressed in the precursor lesions of pancreatic cancer is particularly important for the development of sensitive and specific screening techniques for high-risk patients and for determining the genetic alterations that produce malignancy. Current methods of monitoring patients with predisposing genetic syndromes or a family history of pancreatic cancer include serum CA 19-9 measurements and endoscopic ultrasound (EUS), both of which are of limited value in detecting small pancreatic lesions. For screening purposes, pancreatic juice may provide an ideal specimen, as it potentially has a higher concentration of cancer-related DNA and protein and lacks many normal components of the serum [11].

Recently, a standard system of nomenclature was established to facilitate comparative studies of precursor lesions of pancreatic cancer, now called PanIN (pancreatic intraepithelial neoplasia). Similar to the progression model of colorectal neoplasia, PanINs are thought to progress from flat and papillary mucinous lesions without dysplasia (PanIN-1A and PanIN-1B) to flat or papillary lesions with mild-to-moderate dysplasia (PanIN-2), to carcinoma in situ (PanIN-3), to infiltrating carcinoma [12]. The current molecular progression model of pancreatic cancer associates this histologic progression with the gradual accumulation of genetic alterations in oncogenes, tumor suppressor genes, and mismatch repair genes. Studies of the validity of this model have identified alterations in several genes including *K-ras, HER-2/neu, p16, p53, DPC4*, and *BRCA2.* Point mutations in the *K-ras* oncogene and

Table 1. Overexpressed genes in pancreatic adenocarcinoma: methods of confirmatory testing (as identified by previous studies which confirmed global gene expression findings with individual gene expression analysis).

Gene identified by SAGE and/or microarray study	Cellular component(s) and function(s) (If listed)[a]	Confirmatory test for gene overexpression				References for Confirmatory test data
		Northern Blot	In Situ Hybridization	RT-PCR	Immuno-histochemistry	
14-3-3 sigma, stratifin (SFN)	Cytoplasm and ECM; Cell cycle regulation/G2 cell cycle arrest	*		*	*	6, 10, 26, 34
ABL2	Intracellular; Tyrosine kinase activity			*		9
ADAM9	Integral to PM; Metallopeptidase activity			*		34
ALG-2	Intracellular; Calcium-binding protein			*		37
c-Myc (MYC)	Nucleus; Transcription factor			*	*	9
Catenin, beta (CTNNB1)	PM; Cell adhesion		*			38
Caveolin 2 (CAV2)	Integral to PM			*		34
Cell cycle division 2 (CDC2)	Nucleus; Cell cycle regulation				*	34
CEACAM6	Integral to PM; Cell-cell signaling			*		37
Claudin 1 (CLDN1)	Integral to PM; Tight junction			*		39
Claudin 4 (CLDN4)[b]	Integral to PM; Tight junction			*		26, 34
Connective tissue growth factor (CTGF)	PM; Cell adhesion and cell growth		*			38
DKFZP564G013 protein (RAI14)	Unknown			*		39
Fibronectin 1 (FN1)	Extracellular; Cell adhesion and collagen-binding				*	34
Forkhead box M1 (FOXM1)	Nucleus; Transcription factor			*		34
Galectin 3 (LGALS3)[2]	EC/PM/N; Cell adhesion/IgE binding				*	16
Golgi apparatus protein 1 (GLG1)	Golgi membrane/PM; Receptor binding		*			7
Glyceraldehyde-3-phosphate dehydrogenase (GAPD)[b]	Cytoplasm; Glycolysis	*				20
Heat shock protein 47 (SERPINH1)	Endoplasmic reticulum; Collagen-binding, Serine protease inhibitor				*	39
High mobility group I (Y) (HMG-I (Y))	Intracellular; Regulation of Transcription, Chromatin-binding protein	*				20
Integrin, beta4 (ITGB4)	Integral to PM; Cell adhesion				*	6
Intercellular adhesion molecule-1 (ICAM1)	Integral to PM; Cell adhesion		*			38
IFITM1[b]	Integral to PM; Cell cycle regulation			*		39
KIAA0470 gene product	N/A			*		39
KIAA1265 protein	N/A			*		39
KIAA1363 protein	Catalytic and hydrolase activity			*		39
Keratin 19 (KRT19)	Intermediate filament; Kinesin complex				*	34
Laminin receptor 1 (LAMR1)	Integral to PM, Intracellular; Signal transduction receptor, Cell adhesion	*				20
Lamin B2 (LMNB2) I	Intermediate filament, Nucleus; Function Unknown			*		39
Lipocalin 2 (LCN2)[b]	Cytoplasm; Transporter activity			*	*	16,26
Pleckstrin, family A, member 1 (PLEKHA1)	Phospholipid binding		*			39
MAT-8 (FXYD3)	Integral to PM, Ion transport	*				10
Matrix metalloproteinase 14 (MMP14)	EC/PM; Proteolysis and Peptidolysis		*			38
Mesothelin (MSLN)	PM (GPI-anchored); Cell adhesion		*	*	*	26,31
Microseminoprotein, beta/PSP-94 (MSMB)	EC/N; Molecular function unknown	*				10
Myoferlin/fer-1-like 3 (FER1L3)	Integral to PM; muscle contraction			*		34
NOTCH4	Integral to PM, transcription regulation, Ca-binding, receptor activity			*		8
Osteoblast specific factor 2 (OSF-2)	Extracellular matrix; cell adhesion	*		*		10
Prostate stem cell antigen (PSCA)	PM (GPI-anchored); prostate cancer antigen			*		26,33
RAD51	Intracellular, N; DNA repair			*		9
RAC1	Cell adhesion, cytoskeletal assembly, signal transduction		*			7
RON	N/A					34

Table 1. (Continued).

Gene identified by SAGE and/or microarray study	Cellular component(s) and function(s) (If listed)[a]	Confirmatory test for gene overexpression				References for Confirmatory test data
		Northern Blot	In Situ Hybridization	RT-PCR	Immuno-histochemistry	
S100A4	Ca-binding, invasive growth			*		26, 27, 37
S100A6	Nuclear membrane; Ca-binding, cell cycle regulation					5, 6
S100A10	Ca-binding, signal transduction					34
S100P[b]	Ca-binding, protein-binding			*		6, 7, 16, 34, 39
Sea urchin fascin homolog	N/A					39
Secretory leukocyte proteinase inhibitor (SLPI)	Endopeptidase and serine protease inhibition			*		34
Superoxide dismutase 1 (SOD1)	Cytoplasm; antioxidant activity			*		8
Tetraspan 1 (TSPAN-1)	Integral to PM; cell adhesion, mobility, and proliferation			*		37
Topoisomerase II alpha (TOP2A)	Nucleus; DNA topoisomerase activity				*	39
Trefoil factor 1 (TFF1)[b]	Cell growth and/or maintenance, growth factor activity				*	16
Trefoil factor 2 (TFF2)[b]	Defense response, digestion			*		26
Trefoil factor 3 (TFF3)[b]	EC; defense response digestion				*	16
TGF beta-induced 68 kd protein (TGFBI)	EC; cell adhesion			*		34
Transglutaminase 2 (TGM2)	Ca-binding, transferase activity				*	34
Trop-2	N/A			*		34
XRCC4	DNA double-strand break repair			*		34

EC: extracellular, PM; plasma membrane; N: nucleus; N/A: Gene not found in CGAP Gene Finder.

[a]Information obtained from the CGAP Gene Ontology database.

[b]Differential gene expression has been identified in studies of intraductal papillary mucinous tumors (IPMTs) of the pancreas and may therefore represents a genetic alteration in precursor lesions of pancreatic cancer.

Table 2. Overexpressed genes in pancreatic adenocarcinoma: CGAP data on gene expression *(as identified by previous studies which confirmed global gene expression findings with individual gene expression analysis).*

Gene identified by SAGE and/or microarray study	Cellular component(s) and function(s) (if listed)[a]	CGAP tags per 200,000[b]		Other cancer types expressing the gene, with >63 per 200,000 tags in CGAP (in tissue and/or cell lines)
		Pancreatic cancer	Normal pancreas	
14-3-3 sigma, stratifin (SFN)	Cytoplasm and ECM; cell cycle regulation/G2 cell cycle arrest	32 to 63[B]	<2[B]	None
ABL2	Intracellular; tyrosine kinase activity	<2[C]	<2[C]	None
ADAM9	Integral to PM; metallopeptidase activity	4 to 7[C]	<2[C]	None
ALG-2	Intracellular; calcium-binding protein	2 to 3[C]	2 to 3[C]	None
c-Myc (MYC)	Nucleus; transcription factor	4 to 7[A]	<2[A]	None
Catenin, beta (CTNNB1)	PM; cell adhesion	16 to 31[A]	4 to 7[A]	Lung, colon, peritonerum
Caveolin 2 (CAV2)	Integral to PM	<2[B]	<2[B]	None
Cell cycle division 2 (CDC2)	Nucleus; cell cycle regulation	2 to 3[C]	<2[C]	None
CEACAM6	Integral to PM; cell-cell signaling	32 to 63[C]	<2[C]	Lung
Claudin 1 (CLDN1)	Integral to PM; tight junction	8 to 15[C]	<2[C]	None
Claudin 4 (CLDN4)[c]	Integral to PM; tight junction	64 to 127[B]	<2[B]	Lung, breast, colon
Connective tissue growth factor (CTGF)	PM; cell adhesion and cell growth	32 to 63[A]	16 to 31[A]	Lung, breast, stomach, brain
DKFZP564G013 protein (RAI14)	Unknown	<2[C]	<2[C]	None
Fibronectin 1 (FN1)	Extracellular; cell adhesion and collagen-binding	128 to 255[A]	<2[A]	Lung, peritoneum, brain, ovary
Forkhead box M1 (FOXM1)	Nucleus; transcription factor	2 to 3[A]	<2[A]	None
Galectin 3 (LGALS3)[3]	EC/PM/N;cell adhesion/IgE binding	128 to 255[B]	2 to 3[B]	Breast, brain, lung, colon, skin
Golgi apparatus protein 1 (GLG1)	Golgi membrane/PM; receptor binding	4 to 7[A]	<2[A]	None
Glyceraldehyde-3-phosphate dehydrogenase (GAPD)[c]	Cytoplasm; glycolysis	256 to 512[C]	64 to 127[C]	Brain, lung, breast, stomach, colon peritoneum, ovary, prostate, skin
Heat shock protein 47 (SERPINH1)	Endoplasmic reticulum; collagen-binding, serine protease inhibitor	64 to 127[A]	<2[A]	Lung
High mobility group I (Y) (HMG-I (Y))	Intracellular; regulation of transcription, chromatin-binding protein	N/A	N/A	N/A
Integrin, beta4 (ITGB4)	Integral to PM; cell adhesion	64 to 127[C]	2 to 3[C]	Peritoneum
Intercellular adhesion molecule-1 (ICAM1)	Integral to PM; cell adhesion	32 to 63[A]	<2[A]	None
IFITM1[c]	Integral to PM; cell cycle regulation	32 to 63[A]	<2[A]	Ovary
KIAA0470 gene product	N/A	N/A	N/A	N/A
KIAA1265 protein	N/A	N/A	N/A	N/A
KIAA1363 protein	Catalytic and hydrolase activity	4 to 7[C]	<2[C]	None
Keratin 19 (KRT19)	Intermediate filament; kinesin complex	256 to 512[C]	<2[C]	Breast, stomach, colon, peritoneum, ovary
Laminin receptor 1 (LAMR1)	Integral to PM, intracellular; signal transduction receptor, cell adhesion	128 to 255[C]	128 to 255[C]	Brain, breast, stomach, colon, peritoneum, ovary, prostate, skin
Lamin B2 (LMNB2)	Intermediate filament, nucleus; function unknown	<2[A]	<2[A]	None
Lipocalin 2 (LCN2)[c]	Cytoplasm; transporter activity	4 to 7[C]	<2[C]	None
Pleckstrin, family A, member 1 (PLEKHA1)	Phospholipid binding	2 to 3[C]	4 to 7[C]	None
MAT-8 (FXYD3)	Integral to PM, ion transport	64 to 127[B]	<2[B]	Lung, breast, stomach, colon, prostate
Matrix metalloproteinase 14(MMP14)	EC/PM; proteolysis and peptidolysis	<2[C]	<2[C]	None
Mesothelin (MSLN)	PM (GPI-anchored); cell adhesion	32 to 63[C]	<2[C]	Stomach, peritoneum, ovary
Microseminoprotein, beta/PSP-94 (MSMB)	EC/N; molecular function unknown	<2[C]	<2[C]	Prostate
Myoferlin/fer-1-like 3 (FER1L3)	Integral to PM; muscle contraction	8 to 15[C]	<2[C]	None
NOTCH4	Integral to PM, transcription regulation, Ca-binding, receptor activity	<2[C]	<2[C]	None
Osteoblast specific factor 2 (OSF-2)	Extracellular matrix; cell adhesion	16 to 31[A]	<2[A]	None
Prostate stem cell antigen (PSCA)	PM (GPI-anchored); prostate cancer antigen	32 to 63[B]	<2[B]	None
RAD51	Intracellular, N; DNA repair	<2[C]	<2[C]	None

Table 2. (Continued)

Gene identified by SAGE and/or microarray study	Cellular component(s) and function(s) (if listed)[a]	CGAP tags per 200,000[b]		Other cancer types expressing the gene, with >63 per 200,000 tags in CGAP (in tissue and/or cell lines)
		Pancreatic cancer	Normal pancreas	
RAC1	Cell adhesion, cytoskeletal assembly, signal transduction	16 to 31[B]	2 to 3[B]	None
RON	N/A	N/A	N/A	N/A
S100A4	Ca-binding, invasive growth	128 to 255[C]	<2[C]	None
S100A6	Nuclear membrane; Ca-binding, cell cycle regulation	256 to 512[A]	<2[A]	Lung, colon, peritoneum, skin, brain, breast, prostate
S100A10	Ca-binding, signal transduction	256 to 512[C]	16 to 31[C]	Brain, lung, breast, stomach, colon, peritoneum, ovary
S100P[3]	Ca-binding, protein-binding	64 to 127[C]	<2[C]	Lung, stomach
Sea urchin fascin homolog	N/A	N/A	N/A	N/A
Secretory leukocyte proteinase inhibitor (SLPI)	Endopeptidase and serine protease inhibition	32 to 63[B]	<2[B]	Lung, peritoneum, ovary
Superoxide dismutase 1 (SOD1)	Cytoplasm; antioxidant activity	16 to 31[C]	<2[C]	None
Tetraspan 1 (TSPAN-1)	Integral to PM; cell adhesion, mobility, and proliferation	32 to 63[C]	<2[C]	Prostate
Topoisomerase II alpha (TOP2A)	Nucleus; DNA topoisomerase activity	8 to 15[A]	<2[A]	None
Trefoil factor 1 (TFF1)[c]	Cell growth and/or maintenance, growth factor activity	64 to 127[A]	<2[A]	Breast
Trefoil factor 2 (TFF2)[c]	Defense response, digestion	64 to 127[C]	<2[C]	None
Trefoil factor 3 (TFF3)[c]	EC; defense response, digestion	<2[C]	<2[C]	None
TGF beta-induced 68 kd protein (TGFBI)	EC; cell adhesion	64 to 127[A]	<2[A]	Brain, colon, peritoneum
Transglutaminase 2 (TGM2)	Ca-binding, Transferase activity	16 to 31[A]	8 to 15[A]	Colon, peritoneum
Trop-2	N/A	N/A	N/A	N/A
XRCC4	DNA double-strand break repair	2 to 3[C]	<2[C]	None

[a]Information obtained from the CGAP Gene Ontology database.

[b]The widest gap in CGAP tag data from among the three data options (tissue only, cell lines only, tissue and cell lines) is shown. A; tissue only; B; cell lines only; C; tissue and cell lines; N/A; not found in the CGAP Gene Finder.

[c]Differential gene expression has been identified in studies of intraductal popillary a mucinous tumors (IPMTs) of the pancreas and may therefore represent a genetic alteration in precursor lesions of pancrcatic cancer.

overexpression of *HER-2/neu* are the earliest genetic alterations seen in PanIN-1A and PanIN-1B [13, 14].

Additional support for the progression model of pancreatic cancer has been provided recently by progressively increased immunolabeling of *Ki-67* in PanINs [15]. *Ki-67* is a nuclear protein that correlates well with cellular proliferation and has been used extensively as a proliferation marker for other types of neoplasia. Further studies of genetic alterations in the context of this model may lead to more effective screening and disease-monitoring strategies and may provide clues to the genetic alterations that are responsible for malignancy.

Unfortunately, PanIN lesions are often not clinically detectable and are often inaccessible to biopsy. Intraductal papillary mucinous tumors (IPMTs) of the pancreas are uncommon cystic exocrine pancreatic neoplasms that, in general, have a significantly less aggressive course than ductal adenocarcinomas. The IPMTs have been used recently as a clinically detectable model for studying the early genetic alterations of pancreatic carcinogenesis [16]. Like PanIN, IPMTs can progress from hyperplasia to dysplasia to malignancy. Gene expression profiling of this tumor type at different stages may identify those genes that are differentially expressed in precursor lesions of pancreatic adenocarcinoma [17].

Therapeutic Implications of Gene Overexpression

Knowledge of gene overexpression may be used to develop immunotherapeutic strategies, including the creation of a cell-mediated vaccine or the development of antibody-based immunotherapy. The identification of tumor-specific antigens may allow for the development of a vaccine. Recently, serological analysis of recombinant cDNA libraries (SEREX) has been used to identify overexpressed genes that are immunogenic [18, 19]. The identification of genes overexpressed on the cell surface is particularly useful for the development of antibody-based immunotherapy.

As a therapeutic strategy, molecular targeting may involve inhibition of either the expression or function of the gene product itself or disruption of the signaling transduction pathway in which the gene is involved, with consequent anti-mitogenic action. Examples of the first targeting strategy were recently proposed by a study [20] that identified overexpression of HMG-I (Y) and laminin receptor in pancreatic cancer cell lines derived from metastatic lesions of an orthotopic pancreatic tumor in SCID mice. Inhibition of HMG-I (Y) expression may be accomplished by the introduction of antisense RNA [21], and the laminin receptor may be blocked with monoclonal antibodies to inhibit metastasis [22].

Finally, information on overexpression may be used in the development of gene-therapy strategies. In particular, the promoter regions of overexpressed genes may be incorporated into vectors that deliver "suicide genes" to the tumor cells. These genes, when expressed by the neoplastic cells, act to convert systemically administered prodrugs into active chemotherapeutic agents, thereby localizing the cytotoxic effect to the neoplasm [23].

Clinical Implications of Select Overexpressed Genes

Based on our review of the literature, S100 genes, mesothelin, prostate stem cell antigen, and 14-3-3 sigma/stratifin are candidate genes with promising clinical utility in pancreatic cancer. Confirmatory testing with in-situ hybridization, RT-PCR, and/or immunohistochemistry has consistently shown these proteins to be upregulated.

S100 Genes

Overexpression of several members of the S100 family of calcium-binding proteins has been identified in global gene expression studies and confirmatory RNA and/or protein analyses, including S100A4, S100A6, S100A10, and S100P (Table 1). S100 proteins have been implicated in a variety of intracellular interactions resulting in enzymatic regulation, signal transduction and transcription, calcium homeostasis, and cytoskeletal dysfunction. This final action may confer invasive and metastatic properties. Also, many S100 proteins are released into the extracellular space and may be clinically detectable in the serum and/or pancreatic juice [24]. Additional studies of specific interactions and of signaling pathways involving these genes may lead to the development of anti-metastatic molecular-targeting strategies.

Several S100 proteins, including S100A4 and S100A6, exhibit expression in a variety of other tumors [5, 25] but may still be useful in a serum biomarker panel. Evidence that S100A4 overexpression may be attributable to the stromal response rather than to the neoplastic epithelium has been presented in a recent global gene expression profiling study comparing pancreatic adenocarcinoma and chronic pancreatitis [6]. However, S100A4 overexpression has also been shown in RT-PCR studies of pancreatic cancer cell lines [26, 27] and in immunohistochemical localization performed on pancreatic adenocarcinoma [27], suggesting that overexpression may in fact be associated with the neoplastic epithelium. Additional investigation of S100A4 as a potential aid in diagnosis is warranted. Furthermore, evidence of hypomethylation has been found in S100A4 overexpression [26, 27], and this information may be used to develop therapeutic molecular-targeting strategies.

S100P has recently been identified as a potential biomarker for three reasons. First, like most S100 proteins, it may be secreted into the extracellular space and may, therefore, be detectable in the serum or pancreatic juice. Second, overexpression of the gene has been seen in IPMTs, suggesting that the gene may be expressed at an early stage of carcinogenesis and may therefore be useful for early diagnosis [16]. Third, there is recent evidence obtained by cDNA microarray, SAGE library scanning, and tissue microarray immunohistochemistry that differential expression of S100P is exclusive to neoplastic pancreatic tissue, increasing its specificity as a diagnostic marker [5].

Mesothelin

Mesothelin is a glycosylphosphatidyl inositol-anchored, membrane-bound glycoprotein thought to function in cell adhesion. It is a component of a precursor protein that is proteolytically cleaved to mesothelin and soluble MPFs (mesothelin-related proteins) [28]. Normally, it is expressed in mesothelial cells, with relatively little expression in other normal tissues, including the pancreas. Mesothelin overexpression has been detected in several cancer types, including squamous cell carcinoma of the lung, ovarian cancer, and malignant mesothelioma. Its location in the cell membrane makes it a potential target for antibody-mediated

immunotherapy or the development of a cell-mediated vaccine. Immunotherapeutic targeting of mesothelin in ovarian cancer, malignant mesothelioma, and lung cancer has yielded success in preliminary studies [29, 30].

Mesothelin overexpression has been identified in global gene expression profiling studies of pancreatic cancer, and subsequent in-situ hybridization and immunohistochemical labeling has confirmed that it is expressed in the neoplastic epithelium rather than in the surrounding stroma or nonneoplastic epithelium [31].

Immunohistochemical localization of mesothelin at luminal borders increases the likelihood of its detection, or the detection of MPF, in pancreatic juice or serum [31]. However, its expression in other tumor types decreases its specificity as a serum biomarker, and recent weak immunohistochemical labeling found in a small amount of non-neoplastic pancreatic tissue suggests that it may be most useful alongside other diagnostic studies or in a biomarker panel for pancreatic cancer [31].

Furthermore, mesothelin was not overexpressed in a recent global gene expression profiling study of IPMTs [16], and so it may be of limited use in the detection of precursor lesions. As in S100A4, PSCA (see below), and 14-3-3 sigma, hypomethylation has been identified as a frequent epigenetic alteration of this gene and is highly correlated with overexpression [26].

Prostate Stem Cell Antigen (PSCA)

Prostate stem cell antigen, like mesothelin, is a glycosylphosphatidyl inositol-anchored, membrane-bound glycoprotein and is normally expressed in the prostate, with relatively little expression in other normal tissues, including the pancreas. Overexpression is seen in prostate cancer and is positively correlated with grade, stage, and metastasis in this cancer type [32]. Like mesothelin, it holds promise for new immunotherapeutic strategies.

Immunohistochemical confirmatory tests of global gene expression profiling studies have shown PSCA to be expressed in the neoplastic epithelium rather than in the surrounding stroma or non-neoplastic epithelium. Like mesothelin, its membranous location and expression at luminal borders may allow detection in pancreatic juice and/or serum and may lead to new diagnostic imaging techniques. Also, as in mesothelin, evidence suggests that it may be most effective as a marker when used alongside other diagnostic studies or in a biomarker panel for pancreatic cancer [33]. It was not overexpressed in a recent global gene expression profiling study of IPMTs [16], and so it may be of limited use in the detection of precursor lesions. As in mesothelin, S100A4, and 14-3-3 sigma, hypomethylation has been identified as a frequent epigenetic alteration of this gene and is highly correlated with overexpression [26].

14-3-3 sigma (Stratifin)

14-3-3 sigma/stratifin has been found to act as a tumor-suppressor gene that inhibits cell cycle progression through the G_2 phase. A loss of 14-3-3 sigma expression is seen in breast, lung, head and neck, bladder, and hepatocellular carcinomas. However, global gene expression profiling has identified overexpression of this gene in pancreatic adenocarcinoma. The reverse transcriptase PCR and immunohistochemistry have both been used to confirm overexpression in both cancer tissue and cancer cell lines (Table 1). Recent immunohistochemical labeling of 14-3-3 sigma showed expression primarily in the cytoplasm, with membranous accentuation [34], and the gene has potential as a histologic biomarker.

Findings of overexpression suggest a role in pancreatic carcinogenesis, in contrast to its role as a tumor suppressor in other tissues. An anti-apoptotic role has been previously described for this gene [35]. In addition, the gene may play a role in resistance to chemotherapy, as it was found to be highly expressed in pancreatic cancer cell lines selected for chemotherapeutic resistance [36].

As in mesothelin, S100A4, and PSCA, hypomethylation has been identified as a frequent epigenetic alteration of this gene and is highly correlated with overexpression [26, 34].

Conclusions

Global gene expression profiling is a useful tool for the initial identification of biomarker candidates and potential therapeutic targets. However, these large-scale techniques require subsequent confirmatory testing of mRNA and protein overexpression in order to sufficiently evaluate the proposed gene's sensitivity and specificity for pancreatic cancer. Several genes have been identified in the recent literature as holding promise for the development of new diagnostic and therapeutic strategies, including the S100 family of calcium-binding proteins, mesothelin, prostate stem cell antigen, and 14-3-3 sigma/stratifin.

Acknowledgments

This work was supported by Methodist Hospital Grant 39935 (M.L.), National Institutes of Health grant K08 CA85822 (W.E.F), R13 CA 1011889 (F.C.B.).

References

1. Jemal A, Murray T, Samuels A, et al. Cancer statistics, 2003. CA Cancer J. Clin. 2003;53:5–26
2. Velculescu VE, Zhang L, Vogelstein B, et al. Serial analysis of gene expression. Science 1995;270:484–487
3. Brown PO, Botstein D. Exploring the new world of the genome with DNA microarrays. Nat. Genet. 1999;21(1 Suppl):33–37
4. Pandey A, Mann M. Proteomics to study genes and genomes. Nature 2000;405:837–846
5. Crnogorac-Jurcevic T, Missiaglia E, Blaveri E, et al. Molecular alterations in pancreatic carcinoma: expression profiling shows that dysregulated expression of S100 genes is highly prevalent. J. Pathol. 2003;201:63–74
6. Logsdon CD, Simeone DM, Binkley C, et al. Molecular profiling of pancreatic adenocarcinoma and chronic pancreatitis identifies multiple genes differentially regulated in pancreatic cancer. Cancer Res. 2003;63:2649–2657
7. Crnogorac-Jurcevic T, Efthimiou E, Capelli P, et al. Gene expression profiles of pancreatic cancer and stromal desmoplasia. Oncogene 2001;20:7437–7446
8. Crnogorac-Jurcevic T, Efthimiou E, Nielsen T, et al. Expression profiling of microdissected pancreatic adenocarcinomas. Oncogene 2002;21:4587–4594
9. Han H, Bearss DJ, Browne LW, et al. Identification of differentially expressed genes in pancreatic cancer cells using cDNA microarray. Cancer Res. 2002;62:2890–2896
10. Friess H, Ding J, Kleeff J, et al. Microarray-based identification of differentially expressed growth- and metastasis-associated genes in pancreatic cancer. Cell. Mol. Life Sci. 2003;60:1180–1199
11. Rosty C, Goggins M. Early detection of pancreatic carcinoma. Hematol. Oncol. Clin. North Am. 2002;6:37–52
12. Hruban RH, Adsay NV, Albores-Saavedra J, et al. Pancreatic intraepithelial neoplasia: a new nomenclature and classification system for pancreatic duct lesions. Am. J. Surg. Pathol. 2001;25:579–586

13. Day JD, Digiuseppe JA, Yeo C, et al. Immunohistochemical evaluation of HER-2/neu expression in pancreatic adenocarcinoma and pancreatic intraepithelial neoplasms. Hum Pathol. 1996;27:119–124
14. Hruban RH, van Mansfeld AD, Offerhaus GJ, et al. K-ras oncogene activation in adenocarcinoma of the human pancreas. A study of 82 carcinomas using a combination of mutant-enriched polymerase chain reaction analysis and allele-specific oligonucleotide hybridization. Am. J. Pathol. 1993;143:545–554
15. Klein WM, Hruban RH, Klein-Szanto AJ, et al. Direct correlation between proliferative activity and dysplasia in pancreatic intraepithelial neoplasia (PanIN): additional evidence for a recently proposed model of progression. Mod. Pathol. 2002;15:441–447
16. Terris B, Blaveri E, Crnogorac-Jurcevic T, et al. Characterization of gene expression profiles in intraductal papillary-mucinous tumors of the pancreas. Am. J. Pathol. 2002;160:1745–1754
17. Iacobuzio-Donahue CA, Klimstra DS, Adsay NV, et al. Dpc-4 protein is expressed in virtually all human intraductal papillary mucinous neoplasms of the pancreas: comparison with conventional ductal adenocarcinomas. Am. J. Pathol. 2000;157:755–761
18. Nakatsura T, Senju S, Yamada K, et al. Gene cloning of immunogenic antigens overexpressed in pancreatic cancer. Biochem. Biophys. Res. Commun. 2001;281:936–944
19. Nakatsura T, Senju S, Ito M, et al. Cellular and humoral immune responses to a human pancreatic cancer antigen, coactosin-like protein, originally defined by the SEREX method. Eur. J. Immunol. 2002;32:826–836
20. Tarbe N, Evtimova V, Burtscher H, et al. Transcriptional profiling of cell lines derived from an orthotopic pancreatic tumor model reveals metastasis-associated genes. Anticancer Res. 2001;21:3221–3228
21. Scala S, Portella G, Fedele M, et al. Adenovirus-mediated suppression of HMGI(Y) protein synthesis as potential therapy of human malignant neoplasias. Proc. Natl. Acad. Sci. USA 2000;97:4256–4261
22. Narumi K, Inoue A, Tanaka M, et al. Inhibition of experimental metastasis of human fibrosarcoma cells by anti-recombinant 37-kDa laminin binding protein antibody. Jpn. J. Cancer Res. 1999;90:425–431
23. Yazawa K, Fisher WE, Brunicardi FC. Current progress in suicide gene therapy for cancer. World J. Surg. 2002;26:783–789
24. Donato R. S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int. J. Biochem. Cell. Biol. 2001;33:637–668
25. Ilg EC, Schafer BW, Heizmann CW. Expression pattern of S100 calcium-binding proteins in human tumors, Int. J. Cancer 1996; 68:325–332
26. Sato N, Maitra A, Fukushima N, et al. Frequent hypomethylation of multiple genes overexpressed in pancreatic ductal adenocarcinoma. Cancer Res. 2003;63:4158–4166
27. Rosty C, Ueki T, Argani P, et al. Overexpression of S100A4 in pancreatic ductal adenocarcinomas is associated with poor differentiation and DNA hypomethylation. Am. J. Pathol. 2002;160:45–50
28. Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. Proc. Nat. Acad. Sci. USA 1996;93:136–140
29. Hassan R, Viner JL, Wang QC, et al. Anti-tumor activity of K1-LysPE38QQR, an immunotoxin targeting mesothelin, a cell-surface antigen overexpressed in ovarian cancer and malignant mesothelioma. J. Immunother. 2000;23:473–479
30. Fan D, Yano S, Shinohara H, et al. Targeted therapy against human lung cancer in nude mice by high-affinity recombinant antimesothelin single-chain Fv immunotoxin. Mol. Cancer Ther. 2002;1:595–600
31. Argani P, Iacobuzio-Donahue C, Ryu B, et al. Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE). Clin. Cancer Res. 2001;7:3862–3868
32. Gu Z, Thomas G, Yamashiro J, et al. Prostate stem cell antigen (PSCA) expression increases with high Gleason score, advanced stage and bone metastasis in prostate cancer. Oncogene 2000;19:1288–1296
33. Argani P, Rosty C, Reiter RE, et al. Discovery of new markers of cancer through serial analysis of gene expression: prostate stem cell antigen is overexpressed in pancreatic adenocarcinoma. Cancer Res. 2001;61:4320–4324
34. Iacobuzio-Donahue CA, Maitra A, Olsen M, et al. Exploration of global gene expression patterns in pancreatic adenocarcinoma using cDNA microarrays. Am. J. Pathol. 2003;162:1151–1162
35. Samuel T, Weber HO, Rauch P, et al. The G2/M regulator 14-3-3 sigma prevents apoptosis through sequestration of Bax. J. Biol. Chem. 2001;276:45201–45206
36. Sinha P, Hutter G, Kottgen E, et al. Increased expression of epidermal fatty acid binding protein, cofilin, and 14-3-3-sigma (stratifin) detected by two-dimensional gel electrophoresis, mass spectrometry and microsequencing of drug-resistant human adenocarcinoma of the pancreas. Electrophoresis. 1999;20:2952–2960
37. Ryu B, Jones J, Blades NJ, et al. Relationships and differentially expressed genes among pancreatic cancers examined by large-scale serial analysis of gene expression. Cancer Res. 2002;62:819–826
38. Iacobuzio-Donahue CA, Ryu B, Hruban RH, et al. Exploring the host desmoplastic response to pancreatic carcinoma: gene expression of stromal and neoplastic cells at the site of primary invasion. Am J Pathol. 2002;160:91–99
39. Iacobuzio-Donahue CA, Maitra A, Shen-Ong GL, et al. Discovery of novel tumor markers of pancreatic cancer using global gene expression technology. Am. J. Pathol. 2002;160:1239–1249