Advances in Brief

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)[1]

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Abstract

***Purpose*: Effective new markers of pancreatic carcinoma are urgently needed. In a previous analysis of gene expression in pancreatic adenocarcinoma using serial analysis of gene expression (SAGE), we found that the tag for the mesothelin mRNA transcript was present in seven of eight SAGE libraries derived from pancreatic carcinomas but not in the two SAGE libraries derived from normal pancreatic duct epithelial cells. In this study, we evaluate the potential utility of mesothelin as a tumor marker for pancreatic adenocarcinoma.**

***Experimental Design*: Mesothelin mRNA expression was evaluated in pancreatic adenocarcinomas using reverse-transcription PCR (RT-PCR) and *in situ* hybridization, whereas mesothelin protein expression was evaluated by immunohistochemistry.**

***Results*: Using an online SAGE database (http://www.ncbi.nlm.gov/SAGE), we found the tag for mesothelin to be consistently present in the mesothelioma, ovarian cancer, and pancreatic cancer libraries but not in normal pancreas libraries. Mesothelin mRNA expression was confirmed by *in situ* hybridization in 4 of 4 resected primary pancreatic adenocarcinomas and by RT-PCR in 18 of 20 pancreatic cancer cell lines, whereas mesothelin protein expression was confirmed by immunohistochemistry in all 60 resected primary pancreatic adenocarcinomas studied. The adjacent normal pancreas in these 60 cases did not label, or at most only rare benign pancreatic ducts showed weak labeling for mesothelin.**

***Conclusions*: Mesothelin is a new marker for pancreatic adenocarcinoma identified by gene expression analysis. Mesothelin overexpression in pancreatic adenocarcinoma has potential diagnostic, imaging, and therapeutic implications.**

Introduction

Seven years ago a novel cytokine, MPF,[3] was identified in the culture supernatant of a human pancreatic carcinoma cell line, HPC-Y5 (1). The cDNA encoding this activity was subsequently cloned and found to encode a M_r 69,000 precursor protein, which is proteolytically processed into two components (2, 3). One component, corresponding to the COOH-terminal portion of the precursor, is a membrane-bound, M_r 40,000 protein known as mesothelin. In normal tissues, the expression of mesothelin has subsequently been shown to be largely restricted to mesothelial cells, although immunoreactivity has also been reported in epithelial cells of the trachea, tonsil, fallopian tube, and kidney (4). Mesothelin has been shown to be expressed in squamous carcinomas of the esophagus, lung, cervix, malignant mesothelioma, and ovarian carcinoma, and preliminary studies have shown it to be a promising target for immunotherapy for these malignancies (5, 6). The other component of the M_r 69,000 precursor is a M_r 30,000 NH_2-terminal-secreted protein that corresponds to MPF. Soluble proteins related to MPF have subsequently drawn attention as potential serum tumor markers in patients with ovarian carcinomas that overexpress mesothelin (7). Despite the initial isolation of MPF from a pancreatic cancer cell line, the expression of mesothelin has not been assessed in pancreatic adenocarcinoma, a highly lethal cancer for which promising novel tumor markers and immunotherapy targets are desperately needed.

In a previous analysis of gene expression in pancreatic cancers using SAGE, we identified 47 tags overexpressed and 20 tags underexpressed in pancreatic adenocarcinoma as com-

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[3] The abbreviations used are: MPF, megakaryocyte potentiating factor; SAGE, Serial Analysis of Gene Expression; RT-PCR, reverse transcription PCR; HPDE, human pancreatic duct epithelial; PanIN, pancreatic intraepithelial neoplasia; PL, pancreatic line.

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pared with normal pancreatic ductal epithelium.[4] One of these tags corresponded to mesothelin mRNA and by using SAGE analysis, mesothelin was found to be broadly and highly overexpressed in pancreatic adenocarcinoma. Given the limited distribution of mesothelin in normal tissues, we considered it to be a promising marker for pancreatic cancer and therefore evaluated mesothelin expression in a larger series of pancreatic adenocarcinomas. We now demonstrate, using *in situ* hybridization, RT-PCR, and immunohistochemical labeling, that mesothelin mRNA transcripts and protein are consistently overexpressed in virtually all primary pancreatic adenocarcinomas. This finding has potential diagnostic and therapeutic implications for patients with this often deadly disease.

Materials and Methods

Previous Analysis of Online SAGE Database Using the Student's *t* Test. A large number of normal and neoplastic tissues have now been analyzed by SAGE (8, 9), creating extremely large databases for study. Much of this database is now online and available to the general public[5] (10, 11). As of March 21, 2001, this online database included 94 SAGE libraries and 3,934,110 tags. As reported in detail elsewhere,[4] we have analyzed expression libraries generated by SAGE and identified and narrowed down a set of candidate genes overexpressed in pancreas cancer cell lines as compared with normal pancreatic ductal epithelial cells, using a data reduction algorhythm. Forty-seven overexpressed tags were identified using this approach, and 1 of the 47 was the tag for mesothelin.[4] Mesothelin was selected for further analysis based on its potential use as a tool for diagnosis and treatment (4–6).

Online SAGE Database Analysis Using xProfiler. The online SAGE database has a feature that allows the user to create "virtual Northerns." This tool allows one to view the expression levels of selected SAGE tags in all of the SAGE libraries. Data are presented as "virtual Northerns" allowing the user to simultaneously visualize the levels of gene expression across multiple samples. The mesothelin tag was entered, and a virtual Northern of mesothelin expression in all 94 SAGE libraries was created.

***In Situ* Hybridization for Mesothelin.** A mesothelin-specific riboprobe (bp 1616–2052) was made by first generating a DNA template by PCR with incorporation of a T7 promoter into the antisense or sense primer (12). Following a phenol: chloroform purification of amplified DNA, 200 ng of the DNA template were used to generate either antisense or sense riboprobes by *in vitro* transcription with digoxigenin labeling reagents and T7 polymerase, according to the manufacturer's protocol (Roche Diagnostics, Indianapolis, IN). The methods described by Kadkol *et al.* (13) were then used for *in situ* hybridization on paraffin-embedded tissues. Briefly, sections were deparaffinized in xylene for 5 min, followed by hydration in graded ethanols for 5 min each. Next, sections were digested in 10 μg/ml Proteinase K at 37°C for 30 min, followed by hybridization overnight at 50°C with a 200 ng/ml dilution of antisense or sense riboprobes in mRNA hybridization buffer (DAKO Corp., Carpinteria, CA). The following day, sections were serially washed and incubated with RNase A (Ambion, Austin, TX) and stringently washed twice at 57°C in 2XSSC/50% formamide, followed by one washing at 57°C in 0.8XSSC. Signal amplification was achieved by incubation of the sections with a 1:100 dilution of horseradish peroxidase antiDIG rabbit polyclonal antibody (DAKO), followed by biotinyl-tyramide and secondary strepavidin complex (GenPoint Kit, DAKO). The final signal was developed with 3,3′-diaminobenzidine chromagen (GenPoint Kit, DAKO).

Cell Lines. Human pancreatic cancer cell lines AsPC1, BxPC3, CAPAN1, CAPAN2, CfPAC1, Hs766T, MiaPaca2, and Panc1 were obtained from the American Type Culture Collection (Rockville, MD). Twelve PL cell lines are low-passage pancreatic carcinoma cell lines established in our laboratory. Immortal HPDE cell line obtained after transduction of the *HPV16-E6E7* genes was kindly supplied by Dr. Ming-Sound Taso, University of Toronto, Ontario, Canada. Cells were cultured in RPMI 1640 (Life Technologies, Inc.) supplemented with 100 units/ml of penicillin, 100 μg/ml streptomycin, 4 mM L-glutamine, and 10% fetal calf serum. Cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air.

RT-PCR. Total RNA was extracted from cell lines using Trizol Reagent (Life Technologies). cDNA was synthesized from 1 μg of RNA using the Superscript II kit (Life Technologies) according to the manufacturer's instructions, with oligo$(dT)_{12-18}$ primer. PCR primers were designed from the NCBI GenBank BC003512 sequence to amplify a 226-bp cDNA mesothelin fragment [5′-AACGGCTACCTGGTCCTAG-3′ (sense), 5′-TTTACTGAGCGCGAGTTCTC-3′ (antisense)]. The PCR conditions consisted of an initial denaturation at 95°C for 3 min, followed by 30 cycles of amplification (95°C for 15 s, 58°C for 15 s, and 72°C for 20 s) and a final extension step of 4 min at 72°C. The PCR reaction products were resolved by electrophoresis in a 1.5% agarose gel and stained with ethidium bromide. Loading was controlled by the simultaneous PCR of glyceraldehyde-3-phosphate dehydrogenase cDNA.

Immunohistochemistry for Mesothelin. A series of 60 well-characterized primary invasive pancreatic adenocarcinomas resected at the Johns Hopkins Hospital were selected solely on the basis of tissue availability. For each case, a representative formalin-fixed, paraffin-embedded tissue block containing invasive pancreatic ductal adenocarcinoma and normal tissue was chosen for labeling. Unstained 4-μm sections were then cut from the paraffin block and deparaffinized by routine techniques. The slides were steamed for 20 min in sodium citrate buffer (diluted to 1× from 10× heat-induced epitope retrieval buffer; Ventana-Bio Tek Solutions, Tucson, Arizona). After cooling for 5 min, one slide was labeled with a 1:20 dilution of a mouse monoclonal antibody to mesothelin (clone 5B2; Novocastra, Newcastle upon Tyne, United Kingdom) using the Bio Tek 1000 automated stainer (Ventana). Labeling was detected by adding biotinylated secondary antibodies, avidin-biotin complex, and 3, 3′-diaminobenzidine. Sections were then counterstained with hematoxylin. The extent and intensity of immunolabeling was evaluated jointly by three authors (P. A., R. E. W.,

[4] B. Ryu *et al.*, Identification of differentially expressed genes by serial analysis of gene expression profiling in pancreatic cancer. Cancer Res., in press.

[5] Internet address: www.ncbi.nlm.nih.gov/SAGE.

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Library name	Source	Tags per million	Tag counts	Total tags
SAGE Meso-12	*Primary malignant mesothelioma*	1541	54	35032
SAGE OVT-8	*Primary ovarian cancer*	1370	46	33575
SAGE OVP-5	*Ovarian cancer-cell line*	648	7	10802
SAGE OVT-6	*Primary ovarian cancer*	543	23	42336
SAGE OVT-7	*Primary ovarian cancer*	509	28	54914
SAGE OVCA432-2	*Ovarian cancer-cell line*	349	1	2861
SAGE OV1063-3	*Ovarian cancer-cell line*	25	1	38938
SAGE HOSE 4	*Benign ovary-cell line*	185	9	48413
SAGE ML10-10	*Benign ovary-cell line*	17	1	56943
SAGE Panc 96-6252	*Primary pancreatic cancer*	363	13	35745
SAGE CAPAN2	*Pancreatic cancer-cell line*	129	3	23222
SAGE CAPAN1	*Pancreatic cancer-cell line*	105	4	37926
SAGE Panc 91-16113	*Primary pancreatic cancer*	58	2	33941
SAGE Panc1	*Pancreatic cancer-cell line*	40	1	24879
SAGE SW837	*Colon cancer-cell line*	213	13	60986
SAGE HCT116	*Colon cancer-cell line*	66	4	60322
SAGE Tu98	*Primary colonic cancer*	20	1	49005
SAGE NC2	*Normal colon epithelium*	20	1	49552
SAGE pooled GBM	*Glioblastoma Multiforme*	16	1	61841
SAGE 95-348	*Metastatic Breast cancer*	16	1	60484

Fig. 1 Online SAGE Tag to Gene mapping function (Virtual Northern) demonstrating the distribution of the Hs.155981 (CCCCCTGCAG) tag for mesothelin within the cumulative online SAGE library composed of 3,934,110 tags derived from 94 individual libraries. The tags/million column gives a quantitation of the specific tag's frequency within a specific library, which reflects the level of the corresponding transcript.

and R. H. H.) using a multiobserver microscope. The extent of immunolabeling was categorized into 5 groups: 0% (negative), 1–25% of cells (focal), 26–50% of cells, 51–75% of cells, or 76–100% of cells (diffuse). The intensity of immunolabeling was categorized as weak (+), moderate (++), strong (+++), or intense (++++). All cases demonstrating <25% labeling were categorized as "focal," and all cases showing ≥26% labeling were categorized as "positive." Control tissue (malignant mesothelioma and ovarian serous carcinoma) demonstrated the expected selective tumoral labeling pattern with no stromal labeling at the 1:20 dilution. Labeling was repeated on selected cases using a 1:100 dilution of the primary antibody (see "Results").

Results

Analysis of Online SAGE Database Using the *t* Test. As described in detail elsewhere,[4] our analysis of SAGE databases revealed 47 tags overexpressed and 20 underexpressed in pancreatic cancer as compared with normal pancreatic duct epithelium. The tag corresponding to mesothelin (CCCCCTGCAG) was identified among the 20 most likely to be overexpressed. Specifically, the mesothelin tag was not identified in either of the two normal pancreatic duct epithelium cell lines (HX and H126) but was identified in five of six pancreatic cancer cell lines [SAGE libraries Panc1, CAPAN1, CAPAN2, PL-45 (identical to the PL12 cell line below), and AsPc-1] and in two primary pancreatic adenocarcinomas (library 91-16113 and library 96-6252). The pancreatic cancer cell line Hs766T did not contain a mesothelin tag.

Analysis of Online SAGE Database Using xProfiler. Using the online SAGE Tag to Gene mapping and a database survey that quantitates tags per million (the virtual Northern function), we found that 20 of the 94 libraries in the online database contained the mesothelin tag (Hs.155981; Fig. 1). These 20 libraries could be classified into several categories. Of note, the second largest category consisted of five libraries

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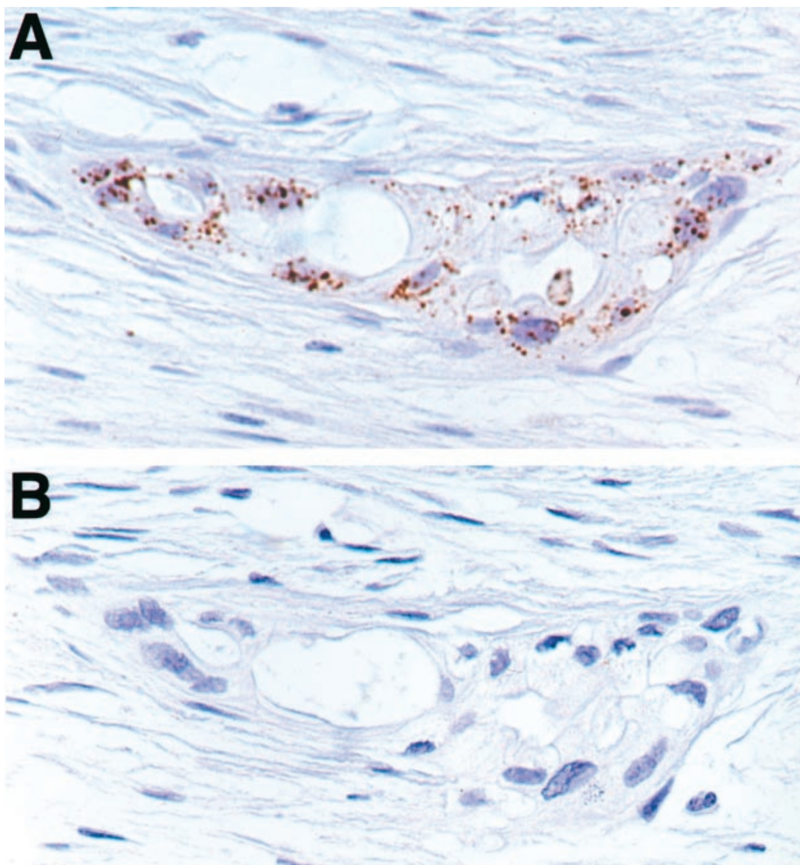

Fig. 2 *In situ* hybridization for mesothelin mRNA transcript using riboprobes. Granular cytoplasmic labeling is present in the invasive carcinoma gland using the antisense probe (*A*) but not with the sense probe (*B*). The surrounding desmoplastic stroma does not label.

derived from pancreatic carcinomas [three from pancreatic cancer cell lines CAPAN1 (105 mesothelin tags/million overall tags), CAPAN2 (129 tags/million), and Panc1 (40 tags/million) and two from primary pancreatic carcinomas 91-16113 (58 tags/million) and 96-6252 (363 tags/million)]. The largest category consisted of nine libraries derived from neoplasms and normal tissues previously known to express mesothelin; namely, ovarian surface epithelial and mesothelial lesions. This grouping included six libraries derived from ovarian carcinomas [three from primary carcinomas OVT-8 (1370 tags/million), OVT-7 (509 tags/million), and OVT-6 (543 tags/million); three from cell lines OVCA432-2 (349 tags/million), OV1063-3 (25 tags/million), and OVP-5 (648 tags/million)]; two libraries derived from benign ovary [ovarian cystadenoma cell line ML10-10 (17 tags/million), normal ovarian epithelium cell line HOSE 4 (185 tags/million)]; and one library derived from a primary malignant mesothelioma Meso-12 (1541 tags/million). The third category was that of colorectal tissues, consisting of three libraries derived from colorectal carcinomas [one from primary colorectal carcinoma Tu98 (20 tags/million), two from colorectal carcinoma cell lines HCT116 (66 tags/million) and SW837 (213 tags/million)], and library NC2 (20 tags/million) derived from normal colorectal epithelium. Two miscellaneous libraries derived from pooled primary glioblastoma multiforme (16 tags/million) and metastatic breast carcinoma (16 tags/million) completed this list. Of note, neither the online library corresponding to short-term cultures of primary pancreatic ductal epithelium (HX and H126) nor the online library derived from pancreatic cancer cell line Hs766T contained a mesothelin tag.

***In Situ* Hybridization.** All four primary resected pancreatic adenocarcinomas examined demonstrated intense granular cytoplasmic labeling with the mesothelin antisense riboprobe. Labeling was not identified in the surrounding stroma using the antisense probe or in any cell type using the sense probe (negative control; Fig. 2). Only weak labeling in the form of rare cytoplasmic granules was noted in rare normal pancreatic ducts with the antisense probe.

RT-PCR. RT-PCR revealed strong mesothelin mRNA expression in 13 of 20 pancreatic cancer cell lines and weak expression in 5 others (Fig. 3). Two pancreatic cancer cell lines (Hs766T and MiaPaca2) did not demonstrate mesothelin transcripts. The immortalized human pancreatic duct epithelial cell line HPDE showed low-level expression of mesothelin. These results are concordant with the online SAGE data. For example, the CAPAN1 and CAPAN2 cell lines, which demonstrated strong mesothelin expression by RT-PCR, each demonstrated >100 mesothelin tags/million. The Panc1 cell line, which showed weak mesothelin expression by RT-PCR, contained fewer mesothelin tags (40) than did CAPAN1 or CAPAN2. Finally, the Hs766T cell line, which did not demonstrate me-

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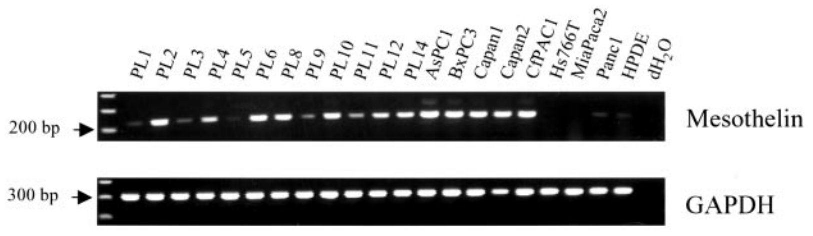

Fig. 3 RT-PCR for mesothelin mRNA of 20 pancreatic cancer cell lines, an immortal HPDE cell line, and a water control. Glyceraldehyde-3-phosphate dehydrogenase serves as an RNA control.

Table 1 Mesothelin immunolabeling summary

	Normal pancreas (n = 60)	PanIN (n = 50)	Infiltrating pancreatic adenocarcinoma (n = 60)
Negative	52 (87%)	40 (98%)	0
Focal	0	0	10 (17%)
Positive	8[a] (13%)	1 (2%)	50 (83%)

[a], weak labeling in scattered atrophic ducts.

sothelin expression by RT-PCR, also lacked the mesothelin tag by SAGE.

Immunohistochemical Labeling. Using the 1:20 dilution, all 60 pancreatic adenocarcinomas labeled for mesothelin (Table 1). Labeling was generally intense (3+ or greater) in 54 of 60 tumors and clearly demarcated neoplastic cells from the surrounding stroma (Fig. 4). Labeling was focal (<25% tumor cells) in 10 cases (17%), diffuse (>75% tumor cells) in 18 cases (30%), and 25–75% of tumor cells labeled in the remaining 32 cancers. When labeling was focal, it tended to be accentuated in single cells surrounded on all sides by stroma, whereas adjacent better-formed malignant glands tended to label less. Additionally, labeling tended to be accentuated at the luminal borders of neoplastic glands, and luminal contents frequently labeled. Normal pancreas in 52 of the 60 cases was essentially nonreactive. In 8 cases, faint "blush" labeling was identified in scattered atrophic pancreatic ducts; this labeling was less intense than that seen in the cancers and readily distinguished from it. In these cases, dilution of the primary mesothelin antibody by 5-fold (to 1:100) eliminated the labeling of nonneoplastic pancreatic ducts, whereas carcinomas retained their labeling. These results were concordant with the *in situ* hybridization results above: all four tumors that demonstrated mesothelin transcripts by *in situ* hybridization labeled strongly for mesothelin protein. Because mesothelin was universally expressed within pancreatic adenocarcinomas, its expression was not a prognostic factor for survival.

A range of PanIN was identified in the sections studied (14). These consisted of 40 duct profiles containing PanINs. Only 2% of the PanINs labeled (Table 1). None of the 19 PanIN-1A lesions, five PanIN-2 lesions, or six PanIN-3 lesions labeled for mesothelin. Among the 11 PanIN-1B lesions identified, only 1 labeled.

Discussion

Pancreatic cancer is the fifth leading cause of cancer death in the United States. This year it has been estimated that 28,000 Americans will be diagnosed with pancreatic cancer and 28,000 will die from it (15, 16). These patients are usually asymptomatic until the tumor has reached an advanced stage, and most patients are not curable with existing therapy. Current methods of early detection are inadequate. Therefore, there is a great need to develop new markers that will increase our ability to diagnose this often deadly cancer earlier.

Using data from an ongoing analysis of gene expression in pancreatic cancer by SAGE, we identified a potential new marker for pancreatic adenocarcinoma, mesothelin. Here, we confirmed the presence of the mesothelin mRNA transcript by *in situ* hybridization and RT-PCR and verified protein expression by immunohistochemical analysis of 60 surgically resected pancreatic ductal adenocarcinomas. These studies were concordant, as overexpression of mesothelin was identified in the majority of pancreatic adenocarcinomas studied by each technique (7 of 8 by SAGE, 4 of 4 by *in situ* hybridization, 18 of 20 by RT-PCR, and 60 of 60 by immunohistochemistry). Of note, one pancreas cancer cell line that did not express mesothelin in either the SAGE database or by RT-PCR, Hs766T, is unusual in that it has a wild-type *p53* gene and shows a gene expression profile that is more similar to that of normal pancreatic duct epithelium by hierarchical cluster analysis (a "normoid" gene expression profile).[4] Hence, mesothelin appears to be consistently and almost universally overexpressed in well-characterized pancreatic adenocarcinoma.

Mesothelin encodes a M_r 40,000 glycoprotein that is attached to the cell membrane by a glycosylphosphatidyl-inositol anchor and is postulated to function in cell adhesion (2). It has been demonstrated to have limited normal tissue distribution by RT-PCR and immunohistochemical studies and is most strongly expressed in normal mesothelium. Mesothelin is synthesized as a M_r 69,000 precursor, from which the NH_2-terminal M_r 30,000 are proteolytically cleaved to produce soluble MPF. The existing data prior to this study support the concept that this gene is not highly expressed in normal pancreas. Normal pancreatic tissue does not express MPF RNA by Northern blotting (3) or mesothelin protein by immunohistochemistry (7). These data are supported by our results. In 52 of the 60 pancreata examined immunohistochemically, the adjacent nonneoplastic pancreatic parenchyma did not label. In 8 cases, atrophic pancreatic parenchyma labeled only weakly, whereas mesothelin transcript was only weakly expressed in an immortalized pancreatic duct epithelial cell line. Hence, mesothelin overexpression in the pancreas appears to be specific for carcinomas. Similar findings, although perhaps less extreme, have been reported in squamous carcinomas of the lung, esophagus, and cervix, where nonneoplastic squamous epithelium in these sites does not label for mesothelin (17, 18).

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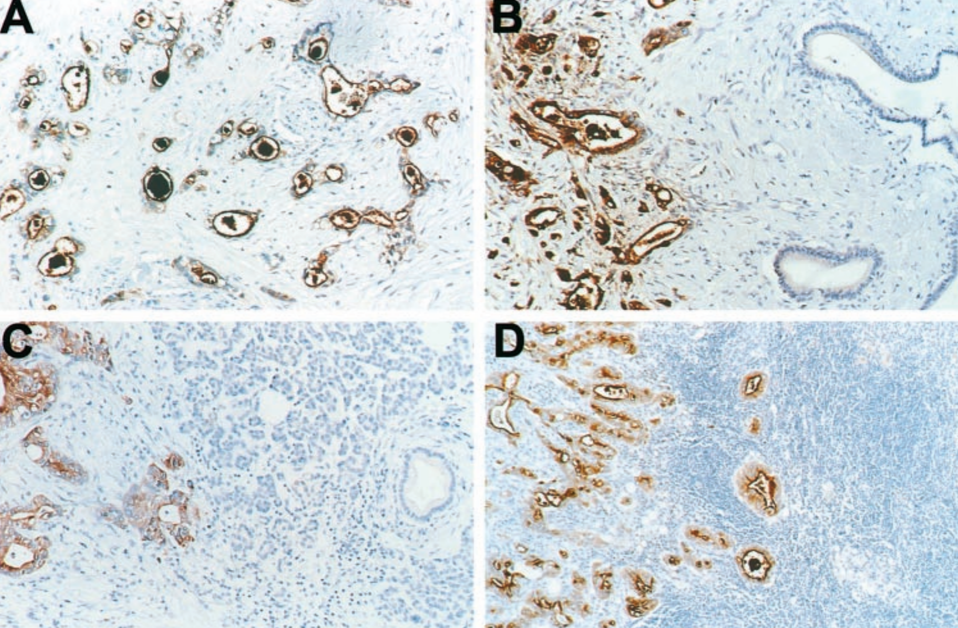

Fig. 4 Immunolabeling for mesothelin protein. There is strong labeling of the neoplastic epithelium and a complete absence of labeling in the nonneoplastic epithelium and stroma (*A–C*). Note the luminal accentuation of mesothelin labeling in *C* and *D*.

The finding of mesothelin overexpression in pancreatic adenocarcinoma has immediate diagnostic applications. Because mesothelin is not strongly expressed in normal pancreas, expression of mesothelin could support the diagnosis of pancreatic adenocarcinoma, particularly in small biopsy or cytopathology samples. However, our identification of mesothelin expression in 2% of the PanINs examined, and weak labeling in rare atrophic pancreatic ducts, indicates that mesothelin labeling, and weak mesothelin labeling in particular, is not specific enough to establish in and of itself the diagnosis of invasive adenocarcinoma in the pancreas.

The finding of mesothelin overexpression in pancreatic adenocarcinoma also has potential screening applications. Tagged antibodies to mesothelin may be useful in diagnostic radiology for *in vivo* imaging of small pancreatic cancer primaries or metastases. Furthermore, the immunohistochemical labeling pattern we identified with mesothelin, one of frequent accentuation at the luminal borders of the malignant glands, raises the possibility that mesothelin and/or MPF may be secreted into pancreatic juice or released into the blood. If so, assays could be devised to detect mesothelin or MPF in the blood, in duodenal and pancreatic fluids, or in stool samples, thereby providing a new marker of pancreatic malignancy. Indeed, MPF-related proteins are readily detectable in the serum of patients with ovarian carcinomas that overexpress mesothelin (7). Moreover, it should be remembered that soluble MPF was initially purified from the supernatant of a pancreatic carcinoma cell line, HPC-Y5. Studies of these fluids from patients with pancreatic adenocarcinoma are currently in progress. Given that mesothelin is known to be overexpressed in other cancers, detection of a soluble form would be unlikely to be specific for pancreatic cancer but could still have utility in specific settings; for example, in evaluating a patient with a strong family history of pancreatic cancer and abnormal radiographic findings in the pancreas. Additionally, soluble mesothelin-related proteins could potentially be part of a panel of markers for pancreatic carcinoma, along with previously used markers such as CA19-9, CEA, and TIMP-1. Although each of these markers is suboptimal, utilization of a panel approach with high cutoffs for each marker has been shown to increase sensitivity without decreasing specificity (19).

The finding of mesothelin overexpression in pancreatic cancer also has potential therapeutic applications. As a cell surface protein, mesothelin is an effective target for immunotoxins used to treat ovarian carcinomas and malignant mesotheliomas. Thus, antibody-based immunotherapy against mesothelin may hold promise as a novel therapy for pancreatic carcinoma (5, 6). Moreover, Jaffee *et al.* have recently demonstrated that cell-mediated immunotherapy can be safe and effective in patients with pancreatic cancer (20). Our findings raise the possibility that mesothelin may also be a rational target for cell-mediated immunotherapy for pancreatic cancer.

In summary, our results confirm that online SAGE databases can be used to identify genes that are differentially expressed between tissues. Previously, we had shown that prostate stem cell antigen is overexpressed in 60% of pancreatic adenocarcinomas using a similar approach (21). We now demonstrate that mesothelin, one of the tags identified in SAGE libraries of pancreatic cancer, is consistently and highly overexpressed in virtually all pancreatic adenocarcinomas at the mRNA and protein levels.

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