Molecular Profiling of Pancreatic Adenocarcinoma and Chronic Pancreatitis Identifies Multiple Genes Differentially Regulated in Pancreatic Cancer

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ABSTRACT

The molecular basis of pancreatic cancer is not understood. Previous attempts to determine the specific genes expressed in pancreatic cancer have been hampered by similarities between adenocarcinoma and chronic pancreatitis. In the current study, microarrays (Affymetrix) were used to profile gene expression in pancreatic adenocarcinoma (10), pancreatic cancer cell lines (7), chronic pancreatitis (5), and normal pancreas (5). Molecular profiling indicated a large number of genes differentially expressed between pancreatic cancer and normal pancreas but many fewer differences between pancreatic cancer and chronic pancreatitis, likely because of the shared stromal influences in the two diseases. To specifically identify genes expressed in neoplastic epithelium, we selected genes more highly expressed (>2-fold, p < 0.01) in adenocarcinoma compared with both normal pancreas and chronic pancreatitis and which were also highly expressed in pancreatic cancer cell lines. This strategy yielded 158 genes, of which 124 were not previously associated with pancreatic cancer. Quantitative-reverse transcription-PCR for two molecules, S100P and 14-3-3, validated the microarray data. Support for the success of the neoplastic cell gene expression identification strategy was obtained by immunocytochemical localization of four representative genes, 14-3-3, S100P, S100A6, and β4 integrin, to neoplastic cells in pancreatic tumors. Thus, comparisons between pancreatic adenocarcinoma, pancreatic cancer cell lines, normal pancreas, and chronic pancreatitis have identified genes that are selectively expressed in the neoplastic epithelium of pancreatic adenocarcinoma. These data provide new insights into the molecular pathology of pancreatic cancer that may be useful for detection, diagnosis, and treatment.

INTRODUCTION

Pancreatic cancer is most frequently adenocarcinoma and has the worst prognosis of all cancers, with a 5-year survival rate of <3%, accounting for the fourth largest number of cancer deaths in the United States (1). Pancreatic cancer occurs with a frequency of around nine patients/100,000 individuals, making it the eleventh most common cancer in the United States. Currently, the only curative treatment for pancreatic cancer is surgery, but only ~10–20% of patients are candidates for surgery at the time of presentation (2), and of this group, only ~20% of patients who undergo a curative operation are alive after 5 years (3). The horrible prognosis and lack of effective treatments for pancreatic cancer arise from several causes. There are currently no effective biomarkers useful for early detection of pancreatic cancer or even to differentiate between pancreatic adenocarcinoma and another major pancreatic disease, chronic pancreatitis. Pancreatic cancer tends to rapidly invade surrounding structures and undergo early metastatic spreading such that it is the cancer least likely to be confined to its organ of origin at the time of diagnosis (4). Finally, pancreatic cancer is highly resistant to both chemotherapy and radiation therapy (4). Currently, the molecular basis for these characteristics of pancreatic cancer is unknown.

Gene expression profiles provide important information about the molecular characteristics of the cancers and can be used to distinguish closely related cancer subtypes (5, 6). Gene profiling can also be used to develop candidate biomarkers (7) and to identify groups of genes involved in specific functional aspects of tumor biology (8). For this reason, several gene profiling approaches have been applied to the investigation of pancreatic cancer, including representational difference analysis (9), serial analyses of gene expression (10, 11), and microarray technologies (12–14). These studies have provided important information and have lead to the discovery of a number of genes that may be useful for pancreas cancer detection, diagnosis, or treatment.

One important consideration in the gene profiling of pancreatic adenocarcinoma is the abundant desmoplastic reaction that occurs in these tumors. These pancreatic tumors are composed of neoplastic cells surrounded by a dense fibrous stroma that contains proliferating fibroblasts, stellate cells, small endothelial-lined vessels, inflammatory cells, and residual parenchymal components of the pancreas. Therefore, comparisons between adenocarcinomas and normal pancreas fail to account for the contribution of stromal elements, and genes identified by these comparisons are not necessarily specific for pancreatic cancer. Chronic pancreatitis, similar to adenocarcinoma, results in lesions containing abundant stroma, which are morphologically identical to that observed in adenocarcinoma. Thus, comparison between adenocarcinomas and chronic pancreatitis allows for the elimination of the stromal contribution, as well as for identification of the genes specifically expressed in neoplastic cells of pancreatic tumors (9, 15, 16).

In the current study, we performed 27 oligonucleotide-directed microarray experiments representing 10 pancreatic tumors, 5 samples of chronic pancreatitis, 5 samples of normal pancreas, and 7 pancreatic cancer cell lines. As expected, initial examination of the data using principle component analysis, clustering, and numerical comparisons indicated that pancreatic tumors were more distinct from normal pancreas than from chronic pancreatitis. Expression profiles were then compared between pancreatic adenocarcinoma, pancreatic cancer cell lines, normal pancreas, and chronic pancreatitis in order to deduct the stromal contribution and more exactly determine the contribution of neoplastic cells. Our selection strategy resulted in a list of 158 genes more highly expressed in both pancreatic adenocarcinoma and pancreatic cancer cell lines compared with noncancerous pancreas. Most (80%) of the genes on this list are novel and have immediate significance as potential diagnostic markers for the differentiation of pancreatic adenocarcinoma and chronic pancreatitis. As an indication of this potential, we selected four genes 14-3-3-σ (stratifin), S100P, S100A6, and β4 integrin for additional investigation as to their expression in neoplastic components of pancreatic adenocarcinoma. This molecular profile of pancreatic adenocarcinoma should help to identify genes involved in pancreatic carcinogenesis, identify
targets for therapy, elucidate clinical biomarkers, and lead to improved understanding of the molecular basis of pancreatic cancer.

MATERIALS AND METHODS

Pancreatic Tissues and Cell Lines. The primary tumors analyzed in this study were derived from the University of Michigan Health System between 1999 and 2001. Samples of chronic pancreatitis came from both the University of Michigan Health System and the Cooperative Human Tissue Network (Midwestern Division Columbus, OH) and conformed to the policies and practices of the University of Michigan Internal Review Board. Samples of normal pancreas were taken from organ donors provided by the Michigan Transplantation Society (four) or from areas outside regions of pathology in surgically resected pancreas (one). All samples were processed in a similar manner. Frozen samples were embedded in OCT-freezing media (Miles Scientific, Naperville, IL), cryotome sectioned (5 μm), and evaluated by routine H&E stains by a surgical pathologist. Areas of relatively pure tumor, chronic pancreatitis, or normal pancreas were microdissected, and these areas were selected for RNA isolation. Pancreatic cancer cell lines BxPC-3, Mia PaCa-2, CFPAC-1, HPAC, MPanc-96, SU.86.86, and SW1990 were obtained from the American Type Culture Collection (Manassas, VA).

Preparation of cRNA and Gene Chip Hybridization. Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA), followed by clean up on a RNeasy spin column (Qiagen, Inc., Valencia, CA) and then used to generate cRNA probes. Preparation of cRNA, hybridization, and scanning of the high-density oligonucleotide microarrays [HuGeneFL arrays (7129 probe sets); Affymetrix, Santa Clara, CA] were performed according to the manufacturer’s protocol (Affymetrix). The preparation of cRNA, hybridization, and scanning of the microarrays were performed according to the manufacturer’s protocols, as reported previously (17).

Data Analysis. Probe intensity values were extracted from the array images using GeneChip 4.0 software (Affymetrix). Each probe set on the HuGeneFL microarray generally consists of 20 coordinated pairs of oligonucleotide features. Within each probe pair, one probe is perfectly complementary (perfect match), whereas the other probe (mismatch) is identical to the complementary probe, except for an altered central base. To obtain an expression measure for a given probe set, the mismatch hybridization values were subtracted from the perfect match values, and the average of the middle 50% of these differences was used as the expression measure for that probe set. In this study, we analyzed 7069 noncontrol probe sets, each of which represents a human transcript. A quantile-normalization procedure was performed to adjust for differences in the probe intensity distribution across different chips. Briefly, we applied a monotone linear spline to each chip that mapped quantiles 0.01 up to 0.99 (in increments of 0.01) exactly to the corresponding quantiles of a tumor chip with low background values as a standard. (Software to obtain probe set intensity measures, quantile-normalize, and compute probe set expression values are available as part of the supplementary material online.)

Statistical Analysis. For statistical tests, we first log transformed each normalized probe-set expression value, x, to log(max(x + 100.0), 100), which we found stabilized the within-group variances between high and low-expression probe sets. To compare normal, tumor, and chronic pancreatitis samples, we performed a one-way ANOVA, modeling the log-transformed values for each probe set as having separate means for each group. Comparison between pairs of groups was performed using the resulting simple contrast tests that are equivalent to ordinary two-sample T tests, except that the variance is estimated using the data from all three groups. We calculated fold changes between groups of samples by first replacing mean expression values < 100 units by 100 in order to avoid negative values or spuriously large fold changes. A PCA4 of the log-transformed data was used to provide a visual depiction of the variation in gene expression. The PCA identifies a set of statistically independent projections, or components, of the expression data. The first principle component captures the greatest fraction of the overall variance in tumor gene expression compared with any other projection. The second principle component captures the greatest fraction of variance subject to being independent of the first projection and so on. Using any two principle components, a pair of coordinates can be determined for each sample. These coordinates can be used to construct a two-dimensional view that reflects the relative locations of samples in the higher dimensional space. Samples that fall close together have more similar gene expression values than samples that fall farther apart. For principal component and clustering analysis, a set of 921 genes was selected without regard to sample origin by asking that the mean of the tissue samples (cell lines excluded) be larger than 100 units, and the SD divided by the mean be >0.80. For PCA, the data were standardized by subtracting the mean and dividing by the SD for the tissue samples in order to give each probe set approximately equal weight.

RT-PCR and Q-RT-PCR. Standard RT-PCR was conducted using total RNA prepared from normal human pancreas, pancreatic adenocarcinomas, and samples of chronic pancreatitis, as described above. Reverse transcription was conducted for 45 min at 45°C from 500 ng of purified total RNA in a 25-μl volume of reverse transcription system reaction mixture by using avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI). Reverse transcription was followed by 35 cycles of standard PCR (1-min denaturation at 94°C, 1-min annealing at 55°C, and 1-min extension at 72°C). All PCR products were verified by sequencing. Primers designed for human S100P (Genbank accession no. X65614) were: forward 5’-ATGACGGAACTAGGACGCGCATGGCCG-3’ and reverse, 5’-GAATCTTGACATTCCATCCTCAGAAC-3’. Primers designed for human 14-3-3σ (Genbank accession no. X57348) were: forward 5’-CCGGATCCCTGTGTCCCCCAGAGCC-3’ and reverse, 5’-CCGAAATTGGCCTGGCCGGACAC-3’. Primers designed for the β-actin (Genbank accession no. BC016045), which was used as a loading control for the RT-PCR reactions, were: forward, 5’-ATGATATCCGGCCTGCTGTC-3’ and reverse, 5’-CGCTCCGGGCTGGTGTGGTAAA-3’. Amplified products were separated on 1.5% agarose gels and visualized by ethidium bromide.

Immunocytochemistry. To identify the cellular source for several of the genes identified in the current study, we used immunocytochemistry. For each gene, at least three different paraffin-embedded tissue blocks containing adenocarcinoma were labeled. Unstained 4-μm sections were deparaffinized with xylene and rehydrated with ethanol. Antigen retrieval was carried out by repetitive 20-s boiling and cooling cycles for a total of 15 min in antigen unmasking solution (Vector Laboratories). Endogenous peroxidase activity was blocked with 6% hydrogen peroxide in methanol, and nonspecific binding sites were blocked with normal donkey serum. Primary antibody (14-3-3σ from Santa Cruz Biotechnology, Santa Cruz, CA; S100P from Research Diagnostics, Inc., Flanders, NJ) diluted (1:250; S100P 1:100) in 2% BSA/0.2% Triton in PBS was added, and samples were incubated overnight at 4°C, after which biotinylated secondary antibody (Santa Cruz Biotechnology) was applied. A biotinylated horseradish peroxidase conjugate (Vector Laboratories) was added and incubated for 30 min at room temperature followed by Vectorstain Elite ABC reagent (Vector Laboratories) and incubation for an additional 30 min at room temperature. Finally, slides were developed with 3,3′-diaminobenzidine substrate (Vector Laboratories), counterstained with hematoxylin, dehydrated with ethanol, and fixed with xylene and mounted.

RESULTS

Gene Expression Profiles Indicate Relationships between Pancreatic Adenocarcinoma, Normal Pancreas, and Chronic Pancreatitis. Comprehensive gene expression profiles were generated using high-density oligonucleotide arrays with 7129 probe sets, which interrogated ~6800 genes. To provide a visual assessment of relationships between the 10 adenocarcinomas, 5 chronic pancreatitis, and 5 normal pancreas samples based on gene expression, we used PCA to locate the three-dimensional view that captured the greatest amount of variability in the data (Fig. 1A). For this analysis, 921 genes were selected on the basis of reasonably high abundance and high sample to sample variability, and the data were standardized to allow each gene to have a nearly equal influence on the outcome. The views generated by PCA indicated substantial differences in gene expression between the three groups of tissue samples (adenocarcinoma, normal, and chronic pancreatitis). A wide margin separated adenocarcinoma samples from normal, and slightly less of a margin separated adenocarcinoma from chronic pancreatitis. Chronic pancreatitis was also

4 The abbreviations used are: PCA, principle component analysis; RT-PCR, reverse transcription-PCR; Q-RT-PCR, quantitative-RT-PCR.
different than normal as four of five samples clustered together, well separated from the normal samples. When data for 7 pancreatic cancer cell lines were plotted on the same axes, it was observed that the cell lines localized in the vicinity of the adenocarcinoma samples (Fig. 1A).

Additional verification of the accuracy of the analysis of relationships between the samples by PCA was obtained using clustering analysis. We used Eisen matrix formats (18) of the 921 genes selected above to investigate the variation in gene expression, show clusters of coordinately expressed genes, and indicate relationships between specimens. The goals of this analysis were 2-fold; first, to see if the mass of data would cluster the samples appropriately; second, to allow visualization of the data in order to examine apparent gene patterns and to see if any unexpected patterns were observed. The sample dendrogram revealed the similarities between the experimental samples (Fig. 1B). In this analysis, the adenocarcinoma samples segregated with the pancreatic cell lines, whereas the normal samples segregated with the chronic pancreatitis samples. Furthermore, four of five chronic pancreatitis samples clustered together, whereas one sample clustered with the normal samples. Using a color-coded scheme derived from the TreeView program, we created a heatmap in which the colors represent relative levels of gene expression with the brightest red indicating the highest level of expression and green depicting low levels or absence of expression. The 921 probe sets were selected by requiring that the mean for tissues (10 adenocarcinomas, 5 chronic pancreatitis, and 5 normal pancreas samples) be >100 units and that the SD divided by the mean be >0.8. For PCA analysis, data were standardized by subtracting the mean and dividing by the SD for the probe set. For average cluster analysis, data were standardized by dividing Affymetrix probe set values by the unweighted mean of the three tissue type averages (adenocarcinomas, chronic pancreatitis, normal pancreas) and taking base 2 logarithms. Negative probe set values were replaced by 1.0. Probe sets and samples were arranged and displayed using Cluster and Treeview software (18).
may be expressed specifically in neoplastic epithelium. A different cluster of genes was highly expressed in both adenocarcinoma and chronic pancreatitis samples but not in either normal pancreas or pancreatic cell lines. Because their expression was not observed in pancreatic cell lines, these genes are likely to originate from stromal elements.

In order to additionally understand the relationship between the various samples, we made numerical comparisons between the genes expressed at higher and lower levels in each group of samples. On the basis of ANOVA, the comparison between pancreatic adenocarcinoma and normal samples yielded 2313 differences in expression levels at \( P < 0.01 \), which is many more than the 71 expected on the basis of chance alone. This same comparison between adenocarcinoma and chronic pancreatitis samples yielded 1086 differences. Using as our criterion a fold-change \( >2.0 \) and \( P < 0.01 \), we compared the numbers of genes differentially expressed in adenocarcinoma and chronic pancreatitis compared with normal pancreas (Table 1). This analysis highlighted the similarities in the distribution of differentially expressed genes in pancreatic adenocarcinoma and chronic pancreatitis. Both diseases shared 322 probe sets identified as being more highly expressed compared with normal pancreas. Furthermore, \(~70\%\) of the probe sets that were either more or less highly expressed compared with normal pancreas in chronic pancreatitis were similarly altered in pancreatic adenocarcinoma. Another striking example of the similarities between the expression profiles of adenocarcinoma and chronic pancreatitis samples is the observation that no gene expressed at higher levels in one was expressed at lower levels in the other, compared with normal pancreas.

**Identification of Genes Differentially Expressed in Pancreatic Adenocarcinoma.** In order to identify genes with expressions that were specific for the neoplastic epithelium of pancreatic adenocarcinoma, we used a strategy involving comparisons between profiles for adenocarcinoma, cancer cell lines, normal pancreas, and chronic pancreatitis samples. The initial step for our strategy was a comparison between genes expressed in pancreatic adenocarcinoma versus chronic pancreatitis and versus normal pancreas (Table 1). This comparison highlights features unique to adenocarcinoma and indicates the existence of 198 probe sets, representing 188 genes, with expression levels that were higher versus both normal and chronic pancreatitis at the 2-fold level (and \( P < 0.01 \) in both comparisons; available in supplementary materials). In order to additionally differentiate between genes arising in neoplastic epithelium and those arising in stroma, we included a comparison of the levels of expression of these 188 genes in pancreatic cancer cell lines to normal pancreas and only accepted genes in which the mean expression levels in the cancer cell lines was at least 2-fold higher than in normal pancreas. This comparison resulted in a \(~16\%\) reduction in the number of selected genes to a final list of 158 genes. To reduce the length of the list for publication purposes, we have restricted the list to genes expressed at \( >3\)-fold in adenocarcinoma compared with both normal and chronic pancreatitis and also in cancer cell lines compared with normal (Fig. 2). This figure indicates the level of expression of each of these 80 genes in the individual samples using a color-coded scheme derived from the TreeView program in which the colors are proportional to the fold change from the median of the tumor, normal, and chronic pancreatitis samples (Fig. 2). The figure also includes the fold increases observed in the means of the adenocarcinoma samples compared with normal pancreas and chronic pancreatitis samples, as well as for the pancreatic cancer cell lines compared with normal pancreas. Genes that had been previously associated with pancreatic cancer are indicated, and a reference is provided. Genes in the list were categorized on the basis of functional data derived from several sources provided by the National Center for Biotechnology Information, including the Mendelian Inheritance in Man site, the Cancer Genome Anatomy Project, and PubMed.

**Validation of Microarray Data and Neoplastic Epithelial Cell Gene Expression Identification Strategy.** As a means of validating that the microarray data accurately reflect mRNA levels, we used RT-PCR and Q-RT-PCR to independently examine mRNA levels for two representative genes, S100P and 14-3-3s, in five separate samples each of normal pancreas, pancreatic adenocarcinoma, and chronic pancreatitis. Affymetrix data for S100P (Fig. 3A) and 14-3-3s (Fig. 3B) indicated that they were highly expressed in all 10 adenocarcinomas but none of the 10 nontumor samples. RT-PCR using high numbers of cycles showed strong bands only in pancreatic tumors (Fig. 3C). In comparison, a weak band was noticed for S100P in some of the normal samples, and 14-3-3s was not present in nontumor samples. Quantitative PCR verified the significant difference between mRNA expression levels in tumor versus nontumor samples (Fig. 3D).

To validate the neoplastic epithelial gene expression identification strategy, we directly determined the cell type in which four genes, S100P, 14-3-3s, \( \beta 4 \) integrin, and S100A6, are expressed in tumors using immunocytochemistry. Expression of these four genes was localized to neoplastic epithelial cells within the tumors (Fig. 4, B, E, H, and K). In contrast, none of the genes were observed to be expressed in stromal cells in chronic pancreatitis (Fig. 4, C, F, I, and L). Likewise, these genes were not expressed in normal acinar or duct cells (Fig. 4, A, D, G, and J). We did note that S100P was expressed to some extent in normal pancreatic islets, which explains the faint bands observed in the RT-PCR analyses. These results support the validity of the microarray selection criteria used in the current study.

To additionally understand the generality of the discovery of these molecules in pancreatic adenocarcinoma, we examined 14-3-3s, S100P, S100A6, and \( \beta 4 \) integrin immunolocalization in paraffin-embedded samples from 28 human adenocarcinoma tumors. Each of these molecules was expressed within the neoplastic epithelial cells of all 28 samples (100\%). These results identify 14-3-3s, S100P, S100A6, and \( \beta 4 \) integrin as potential histological biomarkers for pancreatic adenocarcinoma.

**DISCUSSION**

Despite several earlier studies on gene expression in pancreatic cancer, the majority of the genes identified in this study have not previously been associated with this disease. This is explained by the unique strategy that we used to identify pancreatic cancer-specific

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**Table 1** Numeric distribution of probe sets differentially expressed in pancreatic adenocarcinoma and chronic pancreatitis indicates similarities and differences compared with normal pancreas*

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<td>Lower (743)</td>
<td>No Change (5591)</td>
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<td>CP versus N</td>
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<td>T versus CP</td>
<td>306</td>
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<td>131</td>
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* Data indicate numbers of probe sets that differ between tumor (T), chronic pancreatitis (CP), and normal (N) samples as indicated. “Lower” designates probe sets that were reduced, and “Higher” designates probe sets that were increased at fold-difference \( >2 \) level and \( P < 0.01 \). No significant change\(^2\) indicates that either the differences in expression levels were \( >2\)-fold, or else the \( P \) were \( >0.01 \). Total numbers of probe sets for initial comparisons are given in parentheses.
Fig. 2. Numerous genes are differentially expressed in pancreatic adenocarcinoma. Data from individual samples for genes selected as differentially expressed in pancreatic adenocarcinoma are shown. Data were standardized and displayed in a manner identical to that shown in Fig. 2. The fold changes in the mean values of these genes in pancreatic adenocarcinoma samples compared with the means of normal samples and chronic pancreatitis samples, as well as the fold changes in pancreatic cancer cell lines compared with normal pancreas are also shown. Genes previously described as present in pancreatic cancer or cancer cell lines are indicated by the references. Genes are arranged in functional groupings on the basis of analysis of the relevant literature as indicated.
genes. Our selection strategy compared gene profiles between adeno- carcinoma and both normal pancreas and chronic pancreatitis and used cell lines as the third level of selection. This is a more selective and comprehensive strategy than has been previously applied to pancreatic cancer. We also used oligonucleotide-directed microarray analysis that provides information about a large number of genes and allows detailed statistical analysis for gene selection. The combination of these features allowed the development of a list of genes that has a high probability of being specifically expressed in neoplastic cells within pancreatic adenocarcinoma. These genes have immediate value for their diagnostic potential and also provide targets for additional investigations into the cellular mechanisms of pancreatic cancer.

The selection strategy used in this study was designed, in part, to overcome the obstacle inherent in studies on pancreatic tumors of an abundant desmoplastic reaction. A normal pancreas is composed of primarily acinar cells (~90%), whereas adenocarcinomas include cancer cells, as well as abundant stroma and inflammatory cells. In the current study, many hundreds of genes were found to be differentially expressed between adenocarcinomas and normal pancreas. In contrast, fewer differences were noted between adenocarcinomas and chronic pancreatitis. Chronic pancreatitis, similar to pancreatic cancer, involves a desmoplastic reaction with abundant stroma. Therefore, one explanation for the similarity in expression profiles between tumors and chronic pancreatitis is that many genes expressed in both diseases originate in the stromal components. Previous studies on pancreatic cancer gene expression have not identified the contribution of the stromal elements within pancreatic tumors. Several previous studies have compared pancreatic cancer with normal pancreas (11–13) or even normal colon (10). We compared data from our study to a recent list of 90 genes described from serial analyses of gene expression analysis as being invasion specific in pancreatic cancer (19) and found that ~45% (17 of 38 cosurveyed) of the listed genes were elevated 2-fold or more in the current study compared with normal pancreas, but only ~10% (4/38) were elevated in our study when expression levels in pancreatic adenocarcinoma were compared with those in samples of chronic pancreatitis. Likewise, a comparison of our data to the list of genes described as specifically expressed in pancreatic adenocarcinoma in a recent study using Affymetrix arrays (13) revealed that ~53% (24 of 45 genes cosurveyed) were found to also be increased in chronic pancreatitis in our study. Genes expressed within the desmoplastic stroma may be useful as an indication of the neoplastic-stromal interaction; however, because these genes are not specific for neoplastic cells and are also present in chronic pancreatitis, they are unlikely to be useful either as biomarkers or for specifically understanding the unique molecular basis of this disease. For these reasons, the current analysis excluded these genes.

Laser capture technology provides another means to eliminate the influence of stromal genes as has been recently reported (12). Although this approach makes it difficult to sample from large numbers of tumors, the results are less likely to be compromised by the presence of the stromal elements. However, of the 11 genes recently reported to be specifically up-regulated in neoplastic cells of pancreatic adenocarcinoma using laser capture, ~57% (4 of 7 cosurveyed) of these genes were also highly expressed in chronic pancreatitis as observed in our study. Therefore, these data suggest that even with laser capture technology, it is necessary to compare adenocarcinoma with chronic pancreatitis rather than with normal pancreas in order to discover cancer-specific gene expression.

The influence of the abundant stroma found in pancreatic tumors on the analysis of expression data from pancreatic tumors has been long recognized, and several early studies used various innovative strategies to compensate for this influence. For example, several previous studies used the approach of comparing pancreatic cancer with chronic pancreatitis to eliminate the influence of the stroma (9, 16). Likewise, earlier studies have compared pancreatic tumors with pancreatic cancer cell lines in an effort to restrict the expression profile to genes derived from the malignant epithelial component of the tumor (15). Unfortunately, these early studies were limited by the difficulty of making quantitative comparisons using these early profiling technologies. Thus, when we compared 63 genes described from one earlier study (15) as overexpressed in pancreatic cancer with the results of the current study, we found that 40% (25 of 63 surveyed) were elevated when compared with normal pancreas (with the criterion >2-fold, P < 0.01), but only 17% (11 of 63 surveyed) were elevated when compared with both normal and chronic pancreatitis.
Another more recent innovative approach was to profile fine-needle aspirates from the tumor mass to provide enrichment of tumor cells and avoid stromal influences (20). However, the level of expression of the genes selected as cancer specific in that study was highly variable, and statistically significant differences were not observed between cancer and normal samples. The current study used a quantitative technology that allowed the use of a stringent statistically based selection process.

Comparisons between expression profiles in pancreatic cancer cell lines and normal pancreas have also been used previously as a strategy to restrict analysis of gene expression to neoplastic cells (14). Because cancer cell lines lack stromal elements, this may be a reasonable approach to identification of genes expressed in neoplastic cells. However, cancer cell lines have many important differences compared with primary tumors because of their adaptation to in vitro growth. One important difference between gene expression in the cell lines in vitro and the neoplastic cells within tumors is the lack of the important influence of the stroma on cancer cell gene expression. Furthermore, genes that are important for survival in vivo such as those involved in evading the immune system may be lost in cancer cell lines in vitro, whereas genes that support survival in the in vitro environment may be gained. For these reasons, a direct comparison between cancer cell lines and normal pancreas is unlikely to accurately reflect gene expression in pancreatic adenocarcinoma. Our neoplastic epithelial cell gene identification strategy included the use of pancreatic cancer cell lines as one of several levels of selection. Our rationale was that genes that are more highly expressed in both tumors and cancer cell lines compared with normal pancreas are likely to arise from neoplastic epithelial cells rather than stroma. By including selection based upon expression in the cancer cell lines, our strategy was designed to be highly specific rather than comprehensive. The immunocytochemical localization to the neoplastic epithelium of four of four genes selected on the basis of our strategy provides validation for our neoplastic epithelial gene selection strategy.

The list of genes developed in this study has many potential uses. One use may be to help distinguish between pancreatic adenocarcinoma and chronic pancreatitis. Another use for the list of genes with expressions that are higher in pancreatic adenocarcinoma is in the search for pancreatic cancer biomarkers. The current data provide a large number of candidates for biomarkers. From this initial study, it appears that 14-3-3σ, S100P, S100A6, and β4-integrin may be useful as histological biomarkers for pancreatic adenocarcinoma. Genes specifically expressed in pancreatic cancer may also be targets for therapy. Because of the early nature of this study and the large number of differentially expressed genes, it is presently impossible to properly interpret the global biological significance of all detected differentially expressed genes.

Three of the molecules discovered to be highly specifically ex-

Fig. 4. Validation of the neoplastic epithelial gene selection strategy was performed using immunocytochemistry to localize selected genes in neoplastic cells. Tissues were examined for the presence of 14-3-3σ (A–C), S100P (D–F), S100 A6 (G–I), or β4 integrin (J–L) using antibody localization with horseradish peroxidase. Samples included normal pancreas (A, D, G, and J), pancreatic adenocarcinoma (B, E, H, and K), and chronic pancreatitis (C, F, I, and L). Dark brown staining indicates the presence of the specific antigen. 14-3-3σ was localized exclusively to neoplastic epithelium of pancreatic adenocarcinoma. S100P was also localized to neoplastic cells of pancreatic adenocarcinoma, and no staining was observed in normal duct or acinar cells, although some staining was observed in pancreatic islets (D, arrow). S100A6 was localized to neoplastic epithelial cells and was not observed in sections of normal tissue or chronic pancreatitis. β4-Integrin was localized to neoplastic epithelial cells within pancreatic tumors and was not observed in either normal pancreas or chronic pancreatitis.
pressed in pancreatic adenocarcinoma are members of the S100 protein family, namely S100A6, S100A11, and S100P. S100P has previously been reported in pancreatic cancer in a profiling study (13), as has S100A11 (14). However, expression of these molecules in neoplastic cells was not previously validated by immunocytochemistry. In another recent report, it was suggested that S100A4 is expressed in specifically in pancreatic cancer (7). However, although we observed significantly higher levels of S100A4 in adenocarcinoma compared with normal pancreas, there was no statistically significant difference between the levels in adenocarcinoma compared with chronic pancreatitis. Furthermore, immunocytochemistry confirmed the specific localization of S100A6 and P to cells of the neoplastic epithelium. In contrast, in the current study, S100s A6, A11, and P were more highly expressed in pancreatic cancer than in chronic pancreatitis. In contrast, in the current study, S100s A6, A11, and P were more highly expressed in pancreatic cancer than in chronic pancreatitis. The specific role played by 14-3-3ε in pancreatic adenocarcinoma is unknown but clearly warrant additional investigation.

In the current study, β4 integrin was highly expressed specifically in neoplastic cells of pancreatic adenocarcinoma. Integrins are dimeric proteins composed of noncovalently associated α and β subunits that mediate cellular adhesion and have been found to be important in the progression and spread of cancer. In normal pancreas, the expression of the fibronectin-binding subunit α5; the laminin-binding subunits α2, α3, and α6; and the vitronectin-binding subunit αV have been observed together with the β1, β4, and β5 subunits. In pancreatic neoplastic epithelium, the presence of α2, α3, α5, α6, and αV as well as β1, β3, and β4 have been previously observed (23). However, the differential expression of α2, α3, and β4-integrins in pancreatic adenocarcinoma compared with normal and chronic pancreatitis, as observed in the current study, has not previously been reported. Additional studies will be needed to understand the full significance of these findings.

In summary, comparisons of molecular profiles between pancreatic adenocarcinoma, pancreatic cancer cell lines, normal pancreas, and chronic pancreatitis using oligonucleotide-directed microarrays have allowed the identification of genes specifically altered in the neoplastic epithelium of pancreatic cancer. A key feature of our strategy was the comparison of cancer to chronic pancreatitis in order to avoid the confounding common stromal genes expressed by both diseases to the determination of cancer-specific genes. Furthermore, the comparison of tumor-selective genes to cell lines assured neoplastic specificity because we have validated with four representative molecules by immunocytochemistry. The list of genes specific for the neoplastic pancreatic adenocarcinoma cells developed in this study is highly likely to include some that will be useful as either targets of therapy or as biomarkers and should be very useful in the pursuit of clinically relevant tools for this disease.

ACKNOWLEDGMENTS

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REFERENCES


FUTURE ANNUAL MEETINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

2003 July 11–14, Washington, DC
2004 March 27–31, Orlando, FL
2005 April 16–20, Anaheim, CA

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ADVANCES IN BREAST CANCER RESEARCH: GENETICS, BIOLOGY, AND CLINICAL IMPLICATIONS

October 8–12, 2003
Hyatt Regency Huntington Beach Resort & Spa, Huntington Beach, CA
Chairpersons
Carlos L. Arteaga, Nashville, TN
Lewis A. Chodosh, Philadelphia, PA

NEW DIRECTIONS IN TUMOR ANGIOGENESIS

October 15–19, 2003
Sheraton Chicago, Chicago, IL
Chairpersons
Judah Folkman, Boston, MA
Zena Werb, San Francisco, CA
Peter Carmeliet, Leuven, Belgium

SECOND ANNUAL INTERNATIONAL CONFERENCE ON FRONTIERS IN CANCER PREVENTION RESEARCH

October 26–30, 2003
JW Marriott Desert Ridge Resort, Phoenix, AZ
Chairperson
Raymond N. DuBois, Nashville, TN

AACR-NCI-EORTC INTERNATIONAL CONFERENCE ON MOLECULAR TARGETS AND CANCER THERAPEUTICS

November 17–21, 2003
Hynes Center, Boston, MA
Chairpersons
Charles L. Sawyers, Los Angeles, CA
Edward A. Sausville, Bethesda, MD
Jaap Verweij, Rotterdam, The Netherlands

CALENDAR OF EVENTS

MASCC/ISOO 15th International Symposium of Supportive Care in Cancer, June 18–21, 2003, Berlin, Germany. Contact: Kinga M. Tahy/Julia Boettger, Dachauer Str. 44a, D-80335 Munich, Germany. Phone: 49.89.5490.96.70; Fax: 49.89.5490.96.75; E-mail: mascc@emc-event.com; Website: www.symposium-online.de/mascc.

Advances in Breast Cancer: From Molecular Pathology and Imaging to Therapeutics, June 20–21, 2003, Sutton Place Hotel, Toronto, Ontario, Canada. Contact: Continuing Education, Faculty of Medicine, 500 University Avenue, Suite 650, Toronto, Ontario, M5G 1V7, Canada. Phone: 416.978.2719; Fax: 416.971.2200; E-mail: cc.med@utoronto.ca; Website: www.cme.utoronto.ca.

First Annual Melanoma Research Congress, June 21–24, 2003, Wyndham Franklin Plaza Hotel Philadelphia, PA. Contact: Sandy Parsons, The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104. Phone: 215.898.3959; Fax: 215.898.0980; E-mail: parsons@wistar.upenn.edu.

Fourth International Symposium on Hormonal Carcinogenesis, June 21–25, 2003, Palau de la Musica, Valencia, Spain. Contact: Tandria Price/Dr. Jonathan J. Li, Dept. of Pharmacology, Toxicology and Therapeutics, Mail Stop 1018, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS, 66160-7417. Phone: 913.588.4744; Fax: 913.588.4740; E-mail: tprice@kumc.edu; Website: http://www.kumc.edu/hormonecancers.

6th Cancer Research UK Beatson International Cancer Conference: Cell Signaling and Cancer, July 6–9, 2003, Glasgow, Scotland. Contact: Tricia Wheeler, Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden G61 1BD, Scotland, United Kingdom. Phone: 44(0).141.942.0855; Fax: 44(0).141.330.6426; E-mail: t.wheeler@beatson.gla.ac.uk; Website: http://www.beatson.gla.ac.uk/conf.

First Annual Opinion Leader Summit: Targeted Therapies in the Treatment of Hematological Malignancies, July 9–12, 2003, Kona, HI. Contact: Carl Wilson, S. G. Madison/CBCE, 8445 Freeport Parkway, Suite 680, Irving, TX 75063. Phone: 972.929.1900; Fax: 972.929.1901; E-mail: oguy@sgmadison.com; Website: http://www.sgmadison.com.

Third International Symposium on Translational Research in Oncology, July 23–27, 2003, Fairmont Miramar Hotel, Los Angeles, CA. Contact: Carl Wilson, S. G. Madison/CBCE, 8445 Freeport Parkway, Suite 680, Irving, TX 75063. Phone: 972.929.1900; Fax: 972.929.1901; E-mail: info@thecbce.com; Website: www.sgmadison.com.

12th World Conference on Tobacco or Health: Global Action for a Tobacco Free Future, August 3–8, 2003, Helsinki, Finland. Contact: Conference Secretariat. E-mail: wctoh2003@congcreator.com; Website: www.wctoh2003.org.

Second Annual Symposium on Anti-Signaling Strategies in Human Neoplasia, August 14–16, 2003, Fairmont Hotel, Chicago, IL. Contact: Carl Wilson, S. G. Madison/CBCE, 8445 Freeport Parkway, Suite 680, Irving, TX 75063. Phone: 972.929.1900; Fax: 972.929.1901; E-mail: info@thecbce.com; Website: www.sgmadison.com.
Errata

In the article by C. Giussani et al., entitled “Local Intracerebral Delivery of Endogenous Inhibitors by Osmotic Minipumps Effectively Suppresses Glioma Growth in Vivo,” which appeared in the May 15, 2003 issue of Cancer Research (pp. 2499–2505), the list of authors appeared incorrectly. The correct author list is Carlo Giussani, Giorgio Carrabba, Mauro Pluderi, Valeria Lucini, Marilou Pannacci, Dario Caronzolo, Francesco Costa, Matteo Minotti, Giustino Tomei, Roberto Villani, Rona S. Carroll, Andreas Bikfalvi, and Lorenzo Bello.

In the article by C. D. Logsdon et al., entitled “Molecular Profiling of Pancreatic Adenocarcinoma and Chronic Pancreatitis Identifies Multiple Genes Differentially Regulated in Pancreatic Cancer,” which appeared in the May 15, 2003 issue of Cancer Research (pp. 2649–2657), the list of authors appeared incorrectly. The correct author list is Craig D. Logsdon, Diane M. Simeone, Charles Binkley, Thiruvengadam Arumugam, Joel K. Greenson, Thomas J. Giordano, David E. Misek, Rork Kuick, and Samir Hanash.
Molecular Profiling of Pancreatic Adenocarcinoma and Chronic Panreatitis Identifies Multiple Genes Differentially Regulated in Pancreatic Cancer

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