Evidence for the Importance of Personalized Molecular Profiling in Pancreatic Cancer

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Objective: There is a growing body of evidence that targeted gene therapy holds great promise for the future treatment of cancer. A crucial step in this therapy is the accurate identification of appropriate candidate genes/pathways for targeted treatment. One approach is to identify variant genes/pathways that are significantly enriched in groups of afflicted individuals relative to control subjects. However, if there are multiple molecular pathways to the same cancer, the molecular determinants of the disease may be heterogeneous among individuals and possibly go undetected by group analyses.

Methods: In an effort to explore this question in pancreatic cancer, we compared the most significantly differentially expressed genes/pathways between cancer and control patient samples as determined by group versus personalized analyses.

Results: We found little to no overlap between genes/pathways identified by gene expression profiling using group analyses relative to those identified by personalized analyses.

Conclusions: Our results indicate that personalized and not group molecular profiling is the most appropriate approach for the identification of putative candidates for targeted gene therapy of pancreatic and perhaps other cancers with heterogeneous molecular etiology.

Key Words: cancer diagnostics, personalized cancer profiling, gene expression, microarray

High-throughput molecular profiles (DNA and RNA sequencing, microarray gene expression analyses, etc) are revolutionizing the way cancers are diagnosed, classified, and treated. One well-established approach to identify molecular variants (eg, genetic, epigenetic, or gene expression pattern variants) that may be causally related to complex diseases such as cancer is to identify variant patterns that are significantly enriched in groups of afflicted individuals relative to control subjects. Examples of this approach are the various genome-wide association studies designed to identify disease-causing alleles. While the group approach can, by design, detect genetic or gene expression patterns that are in common among groups of afflicted individuals, genetic variants/molecular patterns that are unique to specific individuals, albeit of potential clinical significance, may go undetected using the group approach. This is likely to be especially true if there are multiple possible molecular paths to the same disease state as is believed to be the case for many, if not all, cancers.

In this study, we were interested in evaluating the impact of using a group versus a personalized approach in the analysis of gene expression profiles of a series of pancreatic cancer patients. We found that the most significant genes/molecular pathways identified among these patients, when analyzed as a group, were substantially different from the significant genes/molecular pathways identified when the analysis was performed on an individual patient basis. Our results are consistent with earlier DNA sequence studies indicating that, on the molecular level, pancreatic cancer is a highly heterogeneous disease, and as a consequence, personalized gene expression profiling is critical to the acquisition of clinically significant information.

MATERIALS AND METHODS

Tissue Collection and Cell Extraction

Patient tissues (Table 1) were collected at St Joseph’s Hospital (Atlanta, Ga) under appropriate institutional review board protocols. Following resection, the tumor tissues were grossly examined by a pathologist and then placed in cryotubes and frozen in liquid nitrogen. Samples were transported on dry ice to Georgia Institute of Technology (Atlanta, Ga), and stored at −80°C.

The tissue samples were examined microscopically, and the histology of ductal adenocarcinoma was verified by a pathologist. Following the examination and verification, tissue samples were embedded in cryomatrix (Shandon, Fisher Scientific, Pittsburg, Pa), and 7-μm frozen sections were cut and attached to uncharged microscope slides. Immediately after dehydration and staining (HistoGene, LCM Frozen Section Staining Kit; Life Technologies, Carlsbad, Calif), slides were processed in an Autopix (Life Technologies) instrument for laser capture microdissection (LCM). For each of the 4 patients, 3 samples from their ductal epithelial tumor cells and 3 samples of their normal ductal epithelial cells were collected. All cells were isolated by LCM to ensure purity of samples. Approximately 30,000 cells were collected for each of the 24 total samples (12 cancer and 12 normal samples).

RNA Extraction and Amplification

PicoPure RNA Isolation Kit (Life Technologies) protocols were followed for RNA extraction from the LCM cells on the Macro LCM caps in 30 μL of extraction buffer. RNA quality was verified for all samples on the Bioanalyzer RNA Pico Chip (Agilent Technologies, Santa Clara, Calif). Total RNA from the above extractions was processed using Ovation Pico WTA System (NuGEN) in conjunction with the Encore BiotinIL Module (NuGEN Technologies, San Carlos, Calif), to produce an amplified, biotin-labeled cDNA suitable for hybridizing to GeneChip Human Genome U133 Plus 2.0 Arrays (Affymetrix, Santa Clara, Calif) following manufacturer’s recommendations.
TABLE 1. Patient Clinical Information at the Time of Surgery and Clinical Outcome at the Time of This Study

| Patient | Sex/Age, y | Tumor Stage | Clinical Outcome (Months After Surgery) |
|---------|------------|-------------|----------------------------------------|
| P1      | Male/77    | T3N0MX II   | No evidence of disease (15)             |
| P2      | Male/69    | T3NXMX II   | Alive with disease (16)                 |
| P3      | Female/55  | T3N1MO II   | No evidence of disease (8)               |
| P4      | Female/67  | T3N1MX II   | Distant metastases (9)                  |

Microarray Data Analysis

We generated 24 individual gene expression profiles from the 3 cancer and 3 normal biological replicate samples of the 4 patients. Affymetrix .CEL files were processed using the Affymetrix Expression Console Software version 1.1 with the Robust Multi-Array Average normalization method. The normalized expression values from all 24 samples were log2 transformed.

Group Analysis

The initial data contained 54,675 probe set expression values from the Affymetrix Human Genome U133 Plus 2.0 chip. For the group analysis, the log2-transformed values were averaged across the 12 cancer and 12 normal samples. An unpaired $t$ test ($P \leq 0.005$) was applied to identify those probe sets (350) that had significantly different expressions between all 12 cancer and all 12 normal samples. These 350 probe sets were used in the group clustering analysis. Of these 350 probe sets, the 287 unique, annotated genes were ranked by fold change (FC). The FC of each gene was calculated by subtracting the average normal value from the average cancer value. Pathway analyses were carried out using the Web-based integrated software suite MetaCore of GeneGO (http://thomsonreuters.com/products_services/science/systems-biology/). Applying the default cutoff $P \leq 0.05$, the 287 genes were found to be enriched for 22 pathways.

Individual Patient Analysis

For the individual patient analysis, the log2-transformed values were averaged across each individual’s cancer and normal replicate samples. From each of the patient’s initial 54,675 probe sets, an unpaired $t$ test ($P \leq 0.005$) was applied to identify 188, 267, 435, and 291 probe sets that had significantly different expression between the cancer and normal replicate samples for each of the patients P1, P2, P3, and P4, respectively. As in the group analysis, these probe sets were used in individual clustering.
analyses (heat maps). Of these, the 148, 211, 351, and 215 unique, annotated genes for P1, P2, P3, and P4, respectively, were ranked according to FC. The FC of each gene was calculated by subtracting the average normal value from the average cancer value for each individual. These genes also were used in the pathway analyses as described above (MetaCore GeneGO software suite). Applying the default cutoff $P \leq 0.05$, the genes were found to be significantly enriched for 15, 17, 25, and 30 pathways in P1, P2, P3, and P4, respectively. For the probe set clusterings (heat maps) in both the group and individual analyses, the log2-transformed values were normalized by Z score statistics.

### Table 2

| Probe Set ID     | Gene Symbol | Fold Change | P       |
|------------------|-------------|-------------|---------|
| 204351_at        | S100P       | 2.514003    | 0.001932|
| 242271_at        | SLC26A9     | 2.178434    | 0.000706|
| 219014_at        | PLAC8       | 1.960672    | 0.001116|
| 239196_at        | ANKRD22     | 1.953161    | 0.004337|
| 239609_s_at      | LPCAT4      | 1.849687    | 0.00536 |
| 205769_at        | SLC27A2     | 1.824208    | 0.001088|
| 238021_s_at      | CRNDE       | 1.768419    | 0.000247|
| 58916_at         | KCTD14      | 1.749122    | 0.000353|
| 213611_at        | AQP5        | 1.690754    | 0.000406|
| 217109_at        | MUC4        | 1.636676    | 0.000648|
| 209277_at        | TFP12       | -2.73483    | 0.001026|
| 223761_at        | FGF19       | -2.67697    | 0.000627|
| 204437_s_at      | FOLR1       | -2.52475    | 0.000341|
| 1554690_a_at     | TACC1       | -2.15521    | 0.000136|
| 214844_s_at      | DOK5        | -2.12474    | 0.004549|
| 216598_s_at      | CCL2        | -2.1185     | 0.000506|
| 223449_at        | SEMA6A      | -2.10883    | 0.001156|
| 207392_s_at      | UGT1B15     | -1.99045    | 0.000764|
| 204151_s_at      | AKR1C1      | -1.95521    | 0.00026 |
| 222901_s_at      | KCNJ16      | -1.93938    | 0.002889|

### RESULTS

#### Group Profiling Identifies Genes and Functional Pathways Previously Implicated in Pancreatic and Other Cancers

**Genes**

In the group profiling, all 12 cancer samples were compared against all 12 normal samples, and 350 probe sets (287 genes) were found to display significant differences in expression ($P < 0.005$). The clustering of these 350 probe sets presented in Figure 1 demonstrates clear separation of the cancer and control samples. However, multiple samples taken from the same patient do not consistently cluster together, indicating heterogeneity within both the cancer and control groups.

Table 2 presents the top 20 most significantly differentially expressed genes (10 most significantly up-regulated and 10 most significantly down-regulated) between the normal and cancer samples as ranked by FC (a complete listing of significantly differentiated genes is presented in Supplemental Tables 1 http://links.lww.com/MPA/A282 and 2 http://links.lww.com/MPA/A283). A summary of the previously documented significance of a representative sampling of these genes is presented in Table 3.

### Pathways

Functional analysis was carried out with the integrated software suite MetaCore of GeneGO (http://thomsonreuters.com/products_services/science/systems-biology/) incorporating the 287 differentially expressed genes. The analysis identified 22 significantly enriched functional pathways ($P \leq 0.05$, Table 4). More than half of the 22 pathways were associated with the immune response (12/22). Oncostatin M appeared in 4 of the 12 immune response pathways. Oncostatin M is a member of a cytokine family that includes leukemia-inhibitory factor, granulocyte colony-stimulating factor, and interleukin 6, and it possesses the ability to inhibit the proliferation of cells in lines derived from several tumor types, including breast carcinoma, ovarian cancer, melanoma, glioma, and lung carcinoma. The 2 most significantly enriched pathways involve androstenedione and testosterone biosynthesis and metabolism (ie, androgen metabolism), both of which have been found significantly altered in pancreatic cancer. Other immune response pathways from the group functional analysis were related to interleukins IL-13, IL-17, and IL-18. Interleukin 13 was previously shown to play a pivotal role in the immunoregulatory pathway of natural killer T cells that suppress tumor immunosurveillance. Although IL-17 seems to have been previously associated with both tumor regression and tumor growth, the specific IL-17 immune response pathway enriched in our analysis contained the protumorigenic gene, CCL2.

### Personalized Profiling Identifies Additional Genes and Functional Pathways Previously Implicated in Cancer

**Genes**

For the personalized profiles, the gene expression data for each individual patient were analyzed similarly to the group profiling analyses. The number of significantly differentially expressed probe sets between cancer and normal replicate samples

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**Note:** The provided text includes a table and a detailed analysis of gene expression data, highlighting significant findings and enriched pathways. The data are presented in a coherent manner, with clear sections for results and discussion.
of each patient ($P \leq 0.005$) varied up to ~2-fold between patients (P1, 188 probe sets; P2, 267 probe sets; P3, 435 probe sets; P4, 291 probe sets). The clustering of these differentially expressed probe sets for each patient is presented as heat maps in Figure 2.

### Table 3. Selective Genes From Table 2 Implicated in Pancreatic and Other Cancers

| Gene Symbol/ Gene Description | Fold Change* | Significance to Pancreatic Cancer and Other Cancers | Ref† |
|------------------------------|--------------|-----------------------------------------------------|------|
| S100P/S100 calcium binding protein P | 2.51 | Implicated in the etiology of prostate and pancreatic cancer | 42,43 |
| ANKRD22/ankyrin repeat domain 22 | 1.95 | Overexpressed in the peripheral blood of pancreatic cancer patients | 44 |
| MUC4/mucin 4 cell surface associated | 1.64 | Overexpressed in pancreatic and other cancers. Facilitates tumor growth and metastasis | 37,45,46 |
| CRNDE/colorectal neoplasia differentially expressed | 1.77 | Elevated expression in colorectal cancer | 47 |
| AQP5/aquaporin 5 | 1.69 | Putative oncogene. Associated with increased proliferation and metastatic potential in breast, lung, non-small cell lung, colorectal cancer, and chronic myelogenous leukemia | 48-53 |
| TFPI-2/tissue factor pathway inhibitor 2 | −2.74 | Down-expression associated with onset of pancreatic and other adenocarcinomas. Regulates extracellular matrix digestion and remodeling. Methylation proposed as a potential biomarker for colorectal cancer | 54-57 |
| FGF19/fibroblast growth factor 19 | −2.68 | Implicated in a variety of cancers | 58 |
| CCL2/chemokine (C-C motif) ligand 2 | −2.12 | Dual role: antitumor activity or tumor growth enhancement | 21 |
| TACC1/transforming, acidic coiled-coil containing protein 1 | −2.16 | Loss of expression associated in ovarian cancer | 59 |

*Fold change: positive (overexpressed in cancer), negative (underexpressed in cancer).
†The list of references is not exhaustive.

A list of the 20 most significantly ($P \leq 0.005$) differentially expressed genes ranked by FC (10 most significantly up-regulated and 10 most significantly down-regulated) between the normal and cancer samples for each individual patient is presented as heat maps in Figure 2.

### Table 4. The 22 Significantly Enriched Pathways ($P \leq 0.05$) of the Differentially Expressed Genes From the Group Analysis (287 genes)

| Group Analysis Pathways | $P$ |
|-------------------------|-----|
| Androstenedione and testosterone biosynthesis and metabolism—p.2 | 0.000342 |
| Androstenedione and testosterone biosynthesis and metabolism—p.2/rodent version | 0.000362 |
| Immune response—oncostatin M signaling via JAK-Stat in mouse cells | 0.01474 |
| Immune response—oncostatin M signaling via JAK-Stat in human cells | 0.01636 |
| Regulation of lipid metabolism—FXR-dependent negative-feedback regulation of bile acids concentration | 0.02527 |
| Cell adhesion—plasmin signaling | 0.02849 |
| Immune response—oncostatin M signaling via MAPK in mouse cells | 0.02849 |
| Immune response—oncostatin M signaling via MAPK in human cells | 0.03009 |
| HIV-1 signaling via CCR5 in macrophages and T lymphocytes | 0.0317 |
| Transport ACM3 in salivary glands | 0.0341 |
| Immune response—IL-13 signaling via JAK-STAT | 0.0357 |
| Immune response—macrophage migration inhibitory factor-induced cell adhesion, migration and angiogenesis | 0.03729 |
| Development—granulocyte-macrophage colony-stimulating factor signaling | 0.04048 |
| Immune response—histamine signaling in dendritic cells | 0.04048 |
| Development—fibroblast growth factor (FGF)—family signaling | 0.04207 |
| Immune response—CCL2 signaling | 0.04366 |
| Chemotaxis CCL2-induced chemotaxis | 0.04524 |
| Immune response—TREM1 signaling pathway | 0.04762 |
| Triacylglycerol metabolism p.1 | 0.04762 |
| Immune response—IL-17 signaling pathways | 0.04841 |
| Immune response—IL-18 signaling | 0.04841 |
| Immune response—CD40 signaling | 0.05235 |
presented in Table 5 (a complete list of all significantly differentially expressed probe sets is presented in Supplemental Tables 1 http://links.lww.com/MPA/A282 and 2 http://links.lww.com/MPA/A283). A summary of the previously documented significance of a representative sampling of these genes is presented in Table 6.

Pathways

As in the group analysis, functional pathway analysis was carried out on all significantly ($P \leq 0.005$) differentially expressed, unique, annotated genes for each patient (P1, 148 genes; P2, 211 genes; P3, 351 genes; P4, 215 genes) to identify functional pathways significantly ($P \leq 0.05$) overrepresented in the cancer samples isolated from each individual patient (Table 7).

Patient 1 (P1)

Five of the 15 most significantly enriched pathways in P1 are associated with the immune response. More specifically, NFAT (nuclear factor of activated T cells) is a major transcriptional regulator in T cells and recently identified as a potent immunoregulator in cancer development and as a potential target for therapeutic manipulation of the immune response in cancer patients. Patient 1 also showed enrichment for the TCR and CD28 signaling pathways. Glutathione metabolism was also identified as a significantly enriched pathway in P1. Glutathione is known to affect the efficacy of antineoplastic interventions mainly through nucleophilic thioether formation or oxidation-reduction reactions. The prevalence of enriched immune response and glutathione metabolism pathways may help account, thus far, for the favorable outcome in P1.

Patient 2 (P2)

Patient 2 displayed pathways that have been implicated strongly in cancer development and invasion. Notch signaling participates in many developmental processes regulating cell differentiation, proliferation, apoptosis, adhesion, epithelial-to-mesenchymal transition, migration, and angiogenesis and can act either as an oncogene or tumor suppressor in a highly context-dependent manner. Cell cycle disruption is a typical feature of cancer cells and results in DNA damage. Cytoskeleton remodeling is required for cancer cell invasion and metastasis, apparent in most cancers. Cell adhesiveness determines the polarity of cells and maintains the cell architecture in tissues. Cell adhesiveness is generally reduced in cancer to allow for invasiveness, extracellular matrix decomposition, and metastasis.

Patient 3 (P3)

Genes in P3 were enriched predominantly for cell cycle regulatory pathways (9 of a total 25 pathways). This is typical for cancer cells at an advanced stage as with P2. Like P1, P3 showed enrichment of interleukin-mediated immune responses and the glutathione metabolism pathway. Interleukin 12 is a powerful coordinator of the innate and adaptive immune responses and has been shown to have promising antitumor effects in murine tumor
models. Interleukin 12 is currently being investigated as a potential therapeutic agent against cancer.

Patient 4 (P4)

The most significantly enriched pathway in P4 was the WNT signaling pathway. The canonical WNT/β-catenin pathway has emerged as a critical regulator in stem cells and has also been associated with cancer in many tissues. For P4, this particular WNT pathway involved the frizzled family receptor 7 (FZD7), which was up-regulated. Up-regulation of FZD7 has been reported in gastric and colorectal cancers. Patient 4 also showed enrichment of apoptotic and survival pathways. In the p53-dependent apoptosis pathway, the BCL2L11 gene (BCL2-like 11-apoptosis facilitator), responsible for cytoplasmic transport of proapoptotic proteins BID, BMF, and BIM, is down-regulated. On the other hand, MCL1 (BCL2-like 10c, apoptosis inhibitory protein) was up-regulated, which is involved in the BAD phosphorylation pathway. This is evidence for deregulation of the apoptosis and survival pathways in P4.
Significant Genes and Pathways in the Personalized Analyses Display Little to No Overlap Among Individual Patients or With Those Identified in the Group Analysis

As shown above, both the group and the personalized analyses identified genes and pathways previously implicated in the onset/progression of pancreatic and a broad spectrum of other cancers. We were next interested in determining the degree of overlap among those genes and pathways identified as significant in each of the individual patient analyses and in the group analysis. Interestingly, we found that the degree of overlap is remarkably low. As shown in Figure 3 (see also Supplemental Tables 1 [http://links.lww.com/MPA/A282] and 2 [http://links.lww.com/MPA/A283]), less than 6.5% (average, 3.3%) of the genes identified

| TABLE 6. Selective Genes From Table 5 Implicated in Pancreatic and Other Cancers |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| P1 PSCA/prostate stem cell antigen | 3.73 | Overexpression in pancreatic adenocarcinoma | 60 | 
| BCAT1/branched-chain amino-acid transaminase 1, cytosolic | 2.96 | Overexpression correlated with clinical outcome of patients with breast, colorectal, neuroendocrine cancer and melanoma | 61-64 | 
| MMP11/matrix metallopeptidase 11 (stromelysin 3) | 2.42 | Expression correlated with aggressiveness of many cancer types | 65-67 | 
| PGC/progastrin | -4.43 | Reduced expression associated with stomach cancer | 68,69 | 
| SULT1E1/sulfotransferase family 1E, estrogen-prefering, member 1 | -3.31 | Down-regulation in prostate and breast cancer tissues and cell lines | 70 | 
| CLDN2/claudin 2 | -3.26 | Reduced expression in most cancers | 71 | 
| CXCL17/chemokine (C-X-C motif) ligand 17 | -2.85 | Loss of expression associated with progression from pancreatic adenoma to pancreatic adenocarcinoma | 72 | 
| BTG2/BTG family, member 2 | -2.77 | Absent in 65% of human breast tumors | 73,74 | 
| VIL1/villin 1 | -2.48 | Loss of expression associated with poorly differentiated colorectal cancers | 75 | 
| NAMPT/nicotinamide phosphoribosyltransferase | -2.23 | Loss of regulation of insulin secretion by pancreatic β cells. Loss of anti-inflammatory and antitumor properties | 76,77 | 
| P2 ODAM/odontogenic ameloblast associated CD55/CD55 molecule, decay accelerating factor for complement (Cromer blood group) | 2.73 | Biomarker for breast cancer | 78 | 
| OLFM4/olfactomedin 4 | 2.63 | Overexpression associated with breast and prostate cancer | 79-81 | 
| ALDH1A2/aldehyde dehydrogenase 1 family, member A2 | 2.26 | Promotes proliferation of pancreatic cancer cells | 82 | 
| ACE2/angiotensin I converting enzyme (peptidyl-dipeptidase A) 2 | -3.54 | Tumor suppressor in pancreatic cancer | 84 | 
| ABCB1/ATP-binding cassette, subfamily B (MDR/TAP), member 1 | -2.3 | Associated with absorption, metabolism, and toxicity of pharmacological agents | 85 | 
| P3 NTS/ neurotensin | 4.05 | Regulates growth of pancreatic cancer cells | 86,87 | 
| WHSC1L1/Wolf-Hirschhorn syndrome candidate 1–like 1 | 1.46 | Overexpressed in breast cancer | 88,89 | 
| CELSR3/cadherin, EGF LAG 7-pass G-type receptor 3 | 3.7 | Involved in contact-mediated communication during cancer progression | 90,91 | 
| ERICH1/glutamate-rich 1 | 3.54 | Associated with higher copy number in pancreatic cancer | 92 | 
| CTRB2/chymotrypsinogen B2 | -5.79 | Overexpression associated with poor prognosis in pancreatic cancer | 93 | 
| CPB1/carboxypeptidase B1 (tissue) | -5.25 | Overexpression associated with poor prognosis in pancreatic cancer | 93 | 
| PNLIPR2/pancreatic lipase–related protein 2 | -5.52 | Overexpression associated with poor prognosis in pancreatic cancer | 93 | 
| CTRLB1/chymotrypsinogen B1 | -5.42 | Down-expression in pancreatic cancer | 94 | 
| REGB1/regenerating isle-derived 1 β | -5.26 | Down-expression in pancreatic cancer | 94 | 
| P4 CA12/carboxy anhydrase XII | -2.93 | Promotes tumor growth and invasion | 95 | 
| ADAM28/ADAM metallopeptidase domain 28 | -2.43 | Overexpressed in many malignant tumors | 96 | 
| DACT1/dapper, antagonist of β-catenin, homolog 1 | -3.64 | Associated with colon cancer progression | 97 | 
| KRT6B/keratin 6B | -3.31 | Overexpressed in triple-negative breast cancer | 98 | 
| ENDRA/endothelin receptor type A | -2.86 | Down-regulation associated with cell invasiveness and carcinogenesis of various cancer types | 99-101 | 

*Fold change: positive (overexpressed in cancer), negative (underexpressed in cancer).
†The list of references is not exhaustive.
TABLE 7. The Significantly (P ≤ 0.05) Enriched Pathways of the Annotated, Unique, Differentially Expressed Genes in P1 (148 Genes, 15 Pathways), P2 (211 Genes, 17 Pathways), P3 (351 Genes, 25 Pathways), and P4 (215 Genes, 30 Pathways)

| P1 Pathway Maps | P | P2 Pathway Maps | P |
|-----------------|---|-----------------|---|
| Immune response—NFAT in immune response | 0.001482 | Development—Notch signaling pathway | 0.0004555 |
| Immune response—CD28 signaling | 0.001942 | Transcription—Sin3 and NuRD in transcription regulation | 0.003978 |
| Cell adhesion—tight junctions | 0.01115 | Cell cycle—nucleocytoplasmic transport of CDK/cyclins | 0.006032 |
| Immune response—TCR and CD28 co-stimulation in activation of nuclear factor (NF) κB | 0.01365 | Development—ligand-independent activation of ESR1 and ESR2 | 0.006414 |
| Neurophysiological process—glutamate regulation of dopamine D1A receptor signaling | 0.01708 | Cytoskeleton remodeling—integrin outside-in signaling | 0.00813 |
| Signal transduction—PKA signaling | 0.02163 | Signal transduction—PKA signaling | 0.00908 |
| Cell adhesion—ECM remodeling | 0.02244 | Development—thrombopoietin signaling via JAK-STAT pathway | 0.01466 |
| Immune response—T cell receptor signaling pathway | 0.02325 | Cell adhesion—endothelial cell contacts by nonjunctional mechanisms | 0.01732 |
| Immune response—immunological synapse formation | 0.02839 | Cell cycle—regulation of G1/S transition (part 2) | 0.02018 |
| Glutathione metabolism | 0.03299 | Neurophysiological process—γ-aminobutyric acid B receptor signaling at postsynaptic sides of synapses | 0.02018 |
| Cardiac hypertrophy—NF-AT signaling in cardiac hypertrophy | 0.03394 | Immune response—inate immune response to RNA viral infection | 0.02322 |
| Glutathione metabolism/human version | 0.03394 | Chemotaxis—leukocyte chemotaxis | 0.0255 |
| Glutathione metabolism/rodent version | 0.03886 | LRRK2 in neurons in Parkinson disease (normal and CF) | 0.03156 |
| Chemotaxis—leukocyte chemotaxis | 0.04404 | G-protein signaling—RhoA regulation pathway | 0.03335 |
| Development—role of nicotinamide in G-CSF–induced granulopoiesis | 0.05239 | Cell cycle—plasmin signaling | 0.03519 |

| P3 Pathway Maps | P | P4 Pathway Maps | P |
|-----------------|---|-----------------|---|
| Cell cycle—chromosome condensation in prometaphase | 4.77E–06 | Development—WNT signaling pathway. Part 2 | 0.0002776 |
| Cell cycle—spindle assembly and chromosome separation | 0.000708 | Apoptosis and survival—p53-dependent apoptosis | 0.000972 |
| Cell cycle—transition and termination of DNA replication | 0.004926 | Mechanisms of CFTR activation by S-nitrosogluthathione (normal and CF) | 0.00372 |
| Proteolysis—putative SUMO-1 pathway | 0.005445 | Cell cycle—nucleocytoplasmic transport of CDK/cyclins | 0.003952 |
| Cell cycle—role of APC in cell cycle regulation | 0.007195 | Mechanism of pioglitazone/metformin and rosiglitazone/metformin cooperative action in diabetes mellitus, type 2 | 0.005167 |
| Androstenedione and testosterone biosynthesis and metabolism p.2 | 0.009241 | Cell cycle—role of 14-3-3 proteins in cell cycle regulation | 0.009688 |
| Cell cycle—the metaphase checkpoint | 0.009991 | DNA damage—ATM/ATR regulation of G2/M checkpoint | 0.0134 |
| Androstenedione and testosterone biosynthesis and metabolism p.2/rodent version | 0.009991 | Cell cycle—transition and termination of DNA replication | 0.01544 |
| Immune response—IL-12–induced interferon γ production | 0.009991 | Development—thrombospondin 1 signaling | 0.01544 |
| Transcription—role of AP-1 in regulation of cellular metabolism | 0.0116 | DNA damage—role of Brca1 and Brca2 in DNA repair | 0.01762 |
| Cell cycle—nucleocytoplasmic transport of CDK/cyclins | 0.01272 | Cell cycle—role of APC in cell cycle regulation | 0.01992 |
| Transport—RAN regulation pathway | 0.0207 | LRRK2 in neurons in Parkinson disease | 0.02111 |
| Cell cycle—sister chromatid cohesion | 0.03027 | Cell cycle—spindle assembly and chromosome separation | 0.02111 |
| Immune response—IL-12 signaling pathway | 0.03289 | Apoptosis and survival—cytoplasmic/mitochondrial transport of proapoptotic proteins Bid, Bmf, and Bim | 0.02233 |
| Glycolysis and gluconeogenesis p.3/human version | 0.03559 | Estradiol metabolism | 0.02358 |

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as significantly differentially expressed between normal and cancer cells isolated from individual patients (personalized profiles) overlap with genes identified as significantly differentially expressed across the combined patient samples (group analysis).

Likewise, there is remarkably little overlap among the individual patients. For example, of the combined number of annotated genes identified as significantly differentially expressed in samples P1 and P2 (148 + 211 = 359), there was less than 1% (2 / 359 = 0.006) overlap. Even between P2 and P3, samples that share the largest number of overlapping genes (8 genes), the degree of overlap is only slightly more than 1% (8 / (211 + 351) = 0.014).

Comparison of the most significantly overrepresented pathways identified in the personalized and group analyses resulted in similar results to the gene analyses; that is, there is relatively little overlap between pathways identified as overrepresented in the group analysis versus the personalized analyses. Furthermore, there is remarkably little overlap in overrepresented pathways among individual patients based on the personalized profiles (Fig. 4; Supplemental Table 3 http://links.lww.com/MPA/A284).

As shown in Figure 4 (see also Supplemental Table 3 http://links.lww.com/MPA/A284), less than 5% (average, 1.7%) of the pathways identified as significantly overrepresented in

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**TABLE 7.** (Continued)

| P3 Pathway Maps | P      | P4 Pathway Maps | P      |
|-----------------|--------|-----------------|--------|
| Glycolysis and gluconeogenesis p.3 | 0.03559 | Estrone metabolism | 0.02358 |
| Cell cycle—initiation of mitosis | 0.03837 | Estradiol metabolism/human version | 0.02486 |
| Immune response—IL-23 signaling pathway | 0.03837 | Estrone metabolism/human version | 0.02486 |
| DNA damage—ATM/ATR regulation of G2/M checkpoint | 0.04124 | Estradiol metabolism/rodent version | 0.02617 |
| Glutathione metabolism/human version | 0.04722 | Cell cycle—regulation of G1/S transition (part 1) | 0.0275 |
| Glycolysis and glucose metabolism (short map) | 0.04904 | Cell adhesion—chemokines and adhesion | 0.0306 |
| Cell cycle—role of SCF complex in cell cycle regulation | 0.0503 | Apoptosis and survival—BAD phosphorylation | 0.03311 |
| Apoptosis and survival—p53-dependent apoptosis | 0.0503 | Signal transduction—AKT signaling | 0.03457 |
| Apoptosis and survival—granzyme A signaling | 0.05346 | Apoptosis and survival—FAS signaling cascades | 0.03606 |
| Cytoskeleton remodeling—Rala regulation pathway | 0.05346 | Development—adiponectin signaling | 0.03758 |
|   | Estrone metabolism/human version | Cytoskeleton remodeling—transforming growth factor, WNT, and cytoskeletal remodeling | 0.03985 |
| NAC-AsPC-1 | 0.04682 | NAC-AsPC-1 | 0.04682 |
| Untitled | 0.04682 | Some pathways of EMT in cancer cells | 0.04716 |

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**FIGURE 3.** Venn diagrams showing the unique, annotated genes identified as significantly differentially expressed in the group analysis and in the personalized analysis(es) of at least 1 patient.
individual patients (personalized profiles) overlap with pathways of genes identified as significantly differentially expressed across the combined patient samples (group analysis). In fact, pathways identified as overrepresented in 2 of the patient samples (P1 and P4) had no overlap with those identified in the group analysis. In addition, there is relatively little overlap among the individual patients. For example, of the pathways identified as significantly overrepresented in samples P1 and P2 (15 + 17 = 32), there was only 6.3% (2/32 = 0.063) overlap. Even between P3 and P4, samples that share the largest number of significantly overrepresented pathways (6 pathways), the degree of overlap is less than 11% (6 / (25 + 30) = 0.109).

The results of the above studies indicate that genes and pathways identified as being most significantly different between normal and cancer samples as determined by the group analysis display little or no overlap with those identified as significant by individual personalized analyses. Likewise, we found little or no overlap in genes and pathways identified as being most significantly different among individual patient samples (personalized analyses).

To determine if our findings were simply an artifact of the relatively high stringency used in identifying significantly differentiated genes ($P \leq 0.005$), we recomputed the degree of overlap between the personalized and group analyses with a variety of cutoff values ranging from 0.05 to 0.001. Although as stringency is reduced, the total number of differentially expressed genes increases as expected, the low overlap between genes identified as significant by the group versus the personalized analyses remained remarkably low (Fig. 5).

To address the possibility that our findings may simply be an artifact of the relatively small number of patients examined in our study, we conducted a similar analysis using data from a previously published microarray gene expression analysis of control and cancer tissue samples isolated from 36 patients.$^{16}$ In
In this earlier study, replicate assays were carried out on 3 patients, allowing us to compare the most significantly differentiated genes as determined by a group analysis (36 patient samples) versus the significantly differentiated genes determined in personalized analyses of 3 patients. Consistent with our previous findings, the results demonstrate remarkably little overlap between genes identified as significant in the group versus personalized analyses (Fig. 6; Supplemental Table 4 http://links.lww.com/MPA/A285).

As shown in Figure 6 (see also Supplemental Table 4 http://links.lww.com/MPA/A285), less than 2% (average, 1.07%) of the genes identified as significantly differentially expressed between normal and cancer cells \( (P < 0.00001) \) isolated from individual patients (personalized profiles) overlap with genes identified as most significantly differentially expressed (top 500 of 17,658 genes significantly differentially expressed, \( P \leq 0.00001 \)) across the combined patient samples. There was no overlap among patients in significantly differentiated genes.

**DISCUSSION**

Molecular profiling is revolutionizing the way we view and treat cancer. Rather than the traditional tissue-of-origin approach to the classification and treatment of the disease, molecular profiling is providing gene-based diagnostics and therapeutics as a realistic alternative. The identification of key genes/pathways associated with various types of cancer is the foundation for both molecular diagnostics and therapeutics.

The group approach to the identification of key genes/pathways involves combining the molecular profiles of collections of samples from diseased patients to identify shared variant profiles that are distinct from those associated with nondiseased controls (eg, see Clarke et al\textsuperscript{13}). Although this can be a productive approach for the detection of biomarkers and potential therapeutic targets for diseases caused by 1 or a few genes, for diseases caused by aberrations in a variety of alternative genes/pathways, the group approach may be less effective.\textsuperscript{34}

Genomes can be profiled with respect to DNA sequence and with respect to gene expression (RNA quantification by microarray or RNAseq analyses, etc). The 2 approaches are complementary in that some functionally significant changes in DNA sequence may not result in changes in gene expression (eg, changes resulting in an altered protein sequence), whereas some changes in gene expression may not be associated with changes in gene sequence (epigenetic changes or changes in a gene’s promoter region, etc). A number of DNA sequence analyses of tumor samples isolated from large numbers of pancreatic cancer patients indicate that, from the gene mutation perspective, pancreatic cancer is a highly heterogeneous disease,\textsuperscript{13,15} suggesting that pancreatic cancer cannot be characterized by a narrowly defined set of mutations across all patients.\textsuperscript{35} In the present study, we were interested in further examining this question by comparing the most significantly differentially expressed genes/pathways between pancreatic cancer and control samples as determined by group versus personalized analyses of the same samples. Toward this end, we used LCM to collect 3 distinct sets (biological replicates) of normal and cancer cells from tissue samples obtained from 4 pancreatic patients. In addition, we reanalyzed data from a previous gene expression analysis of 36 pancreatic patients\textsuperscript{16} and compared the most significantly differentiated genes/pathways as determined by the group analysis relative to the most significantly differentiated genes/pathways as determined by personalized analyses of 3 patients for which replicate microarray assays were performed.

Our results consistently demonstrated little to no overlap between genes/pathways identified in the group analyses relative to those identified in the personalized analyses. For example, consistent with earlier reports,\textsuperscript{36} our group analysis identified MUC4 as one of the most significantly differentiated.
expressed genes between the normal and pancreatic cancer samples (Table 2). Indeed, MUC4 has recently been proposed as a prime candidate for targeted drug therapy in pancreatic cancer.37 In our personalized analyses, however, MUC4 was identified as significantly overexpressed in only 1 of the 7 patients examined suggesting that MUC4 therapy would likely not be effective for the majority of the patients examined in our study. Conversely, many of the genes identified as being significantly differentially expressed in individual patients (personalized profiles) were not identified as significant in the group analysis. For example, the most significantly differentially expressed gene in the cancer samples isolated from P1 is PSCA (prostate stem cell antigen). Interestingly, a monoclonal antibody against PSCA is currently being tested in clinical trials for prostate cancer (prostate stem cell antigen). Interestingly, a monoclonal antibody against PSCA is currently being tested in clinical trials for prostate cancer. For example, the most significantly differentially expressed gene in the cancer samples isolated from P1 is PSCA (prostate stem cell antigen). Interestingly, a monoclonal antibody against PSCA is currently being tested in clinical trials for prostate cancer.

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