OPEN

Evidence for the Importance of Personalized Molecular Profiling in Pancreatic Cancer

Loukia N. Lili, PhD, Lilya V. Matyunina, PhD,* L. DeEtte Walker, PhD,* George W. Daneker, MD,†
and John F. McDonald, PhD**

Objectives: 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

(*Pancreas* 2014;43: 198–211)

High-throughput molecular profiles (DNA and RNA sequencing, microarray gene expression analyses, etc) are revolutionizing the way cancers are diagnosed,[1–4] classified,[5,6] and treated.[7–9] 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.[10,11] 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.[12]

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,[13–15] 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	T3N1M0 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 $\log_2$ 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 $\log_2$-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 $\log_2$-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
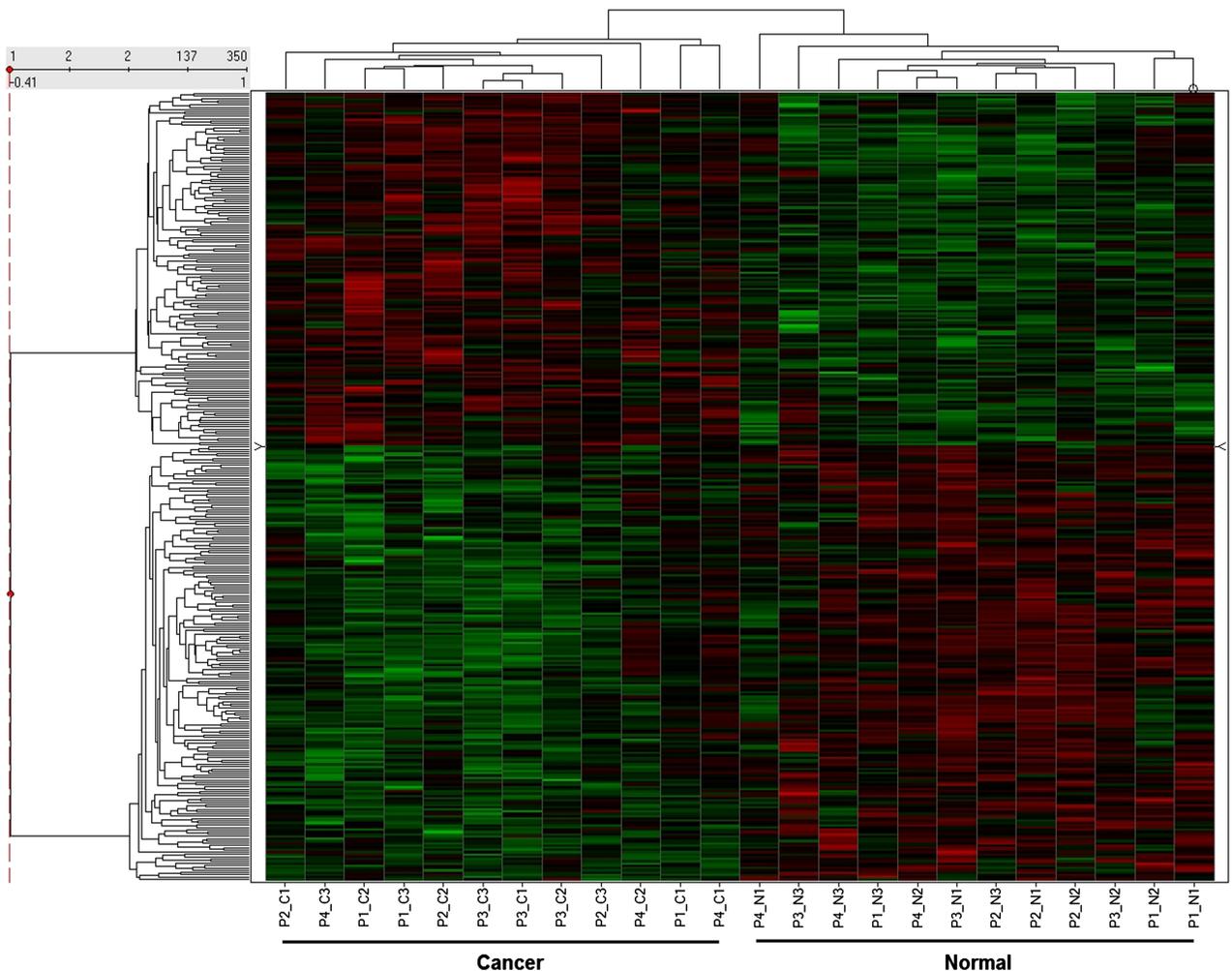
FIGURE 1. Hierarchical clustering of the 350 probe sets that display a significant ($P \leq 0.005$) difference in gene expression among all cancer and normal samples. The heat map was generated by z score normalization of $\log_2$ expression values from the Affymetrix HG U133 Plus 2.0 chip. Patients are denoted as P1, P2, P3, and P4 with their associated cancer and normal biological replicate samples (ie, C1, C2, C3 and N1, N2, N3, respectively).

TABLE 2. The 10 Most Significantly Up-regulated and the 10 Most Significantly Down-regulated Genes in Cancer Resulting From the Group Analysis of the 287 Unique, Annotated, Significantly Differentially Expressed Genes

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	0.000116
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	TFPI2	−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_x_at	UGT2B15	−1.99045	0.000764
204151_x_at	AKR1C1	−1.95521	0.00026
222901_s_at	KCNJ16	−1.93938	0.002889

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 $\log_2$-transformed values were normalized by Z score statistics.

Analysis of Data From the Previously Published Study of Badea et al

Seventy-eight Affymetrix .CEL files were downloaded from the GEO Omnibus database with accession number GDS4103.[16] The files were processed using the Affymetrix Expression Console Software version 1.1 with the Robust Multi-Array Average normalization method, and the normalized expression values were $\log_2$ transformed, similarly to our sample analysis. All the 78 samples from 36 patients were used for the group analysis. For the individual analysis, the available 2 replicate cancer and 2 replicate normal samples from 3 patients were used (herein referred to as patients P5, P6, and P7; 12 samples total). Both the group and the individual analyses were performed using the methods described above. Because technical replicates (multiple assays of the same biological patient sample) rather than biological replicates (assays of multiple biological samples from the same patient), as assayed in our study, were used in the study of Badea et al,[16] the number of significantly differentiated genes at $P \leq 0.005$ was more than an order of magnitude greater than that in our study. Thus, we used an unpaired t test with a more stringent cutoff ($P \leq 0.00001$) than in our analysis to keep the number of significantly differentiated genes comparable to our study. Using this criterion, 17,658 significantly differentially expressed probe

sets were detected, of which the 500 (330 annotated, unique genes) most significant were used for further analysis. For the individual gene analysis, the same unpaired t test with $P \leq 0.00001$ identified 12, 37, and 22 significant probe sets (12, 29, and 20 annotated, unique genes) in patients P5, P6, and P7, respectively.

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 ($P \leq 0.005$). 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 significant 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.[17] 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.[18] 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.[19] Although IL-17 seems to have been previously associated with both tumor regression and tumor growth,[20] the specific IL-17 immune response pathway enriched in our analysis contained the protumorigenic gene, CCL2.[21]

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 identically to the group profiling analyses. The number of significantly differentially expressed probe sets between cancer and normal replicate samples

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†
Group analysis	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.

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.

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

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
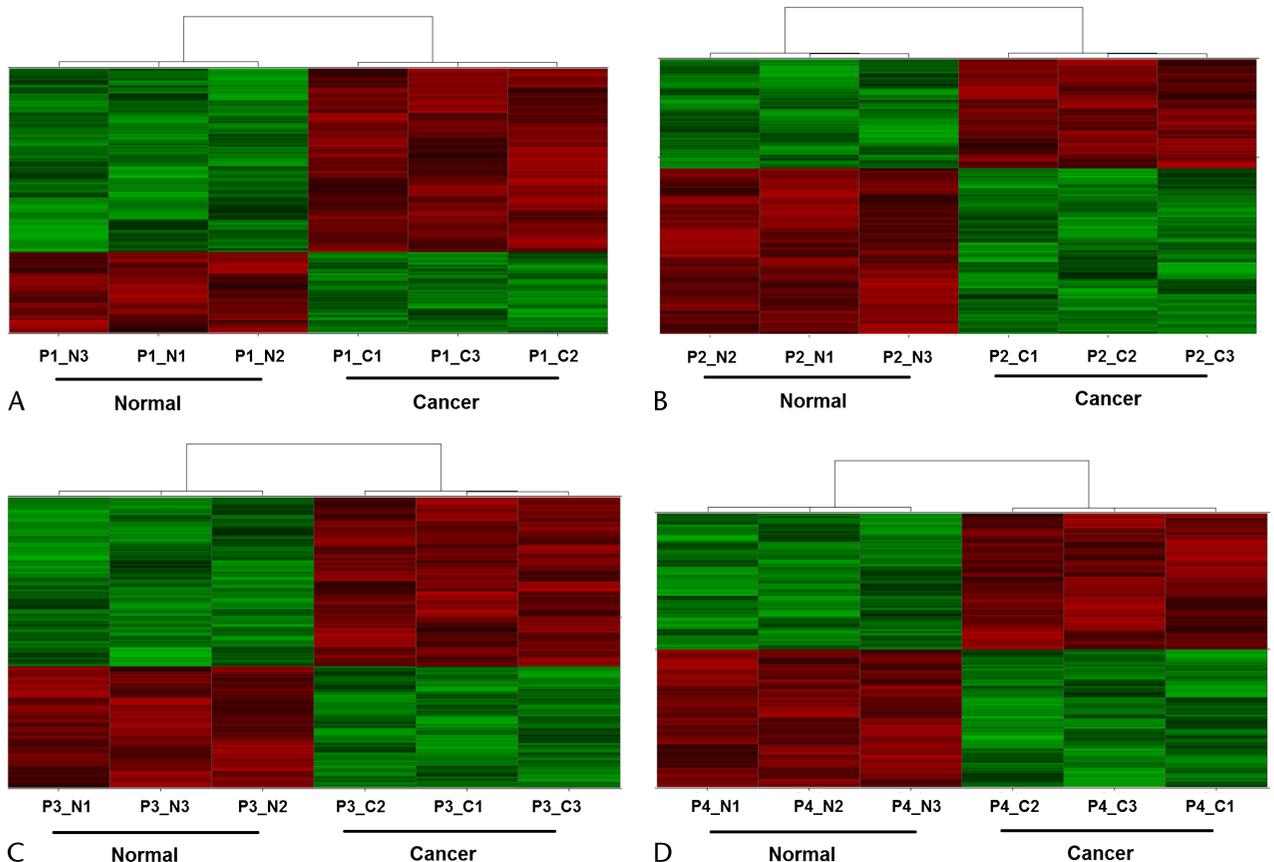
FIGURE 2. Supervised clustering of probe sets displaying a significant ($P \leq 0.005$) difference in expression between normal and cancer samples for patients P1 (A), P2 (B), P3 (C), and P4 (D). The heat map was generated by z score normalization of $\log_2$ expression values from the Affymetrix HG U133 Plus 2.0 chip. Patients are denoted as P1, P2, P3, and P4 with their associated cancer and normal biological replicate samples (ie, C1, C2, C3 and N1, N2, N3, respectively).

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.[22] 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.[23] 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.[24] Cell cycle disruption is a typical feature of cancer cells and results in DNA damage.[25] Cytoskeleton remodeling is required for cancer cell invasion and metastasis, apparent in most cancers.[26] Cell adhesion 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.[27]

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

TABLE 5. The 10 Most Significantly Up-regulated and 10 Most Down-regulated Genes (*P* ≤ 0.005) Between Normal and Cancer Samples for Each Patient From the Personalized Profiling Analysis (Fig. 2)

Probe Set ID (Individual P1)	Gene Symbol	Fold Change	*P*	Probe Set ID (Individual P2)	Gene Symbol	Fold Change	*P*
205319_at	PSCA	3.732981	0.000613	220576_at	PGAP1	3.318862	0.001723
226517_at	BCAT1	2.959422	5.25E−05	217110_s_at	MUC4	3.103023	3.01E−05
1555294_a_at	ERC1	2.923769	0.000932	220133_at	ODAM	2.726326	0.003759
226325_at	ADSSL1	2.798377	0.001668	1567679_at	SNORA74A	2.636617	0.001267
228010_at	PPP2R2C	2.667305	0.001297	201926_s_at	CD55	2.627469	0.000945
52255_s_at	COL5A3	2.660518	0.001885	216504_s_at	SLC39A8	2.436613	0.002912
203877_at	MMP11	2.417413	0.002271	238022_at	CRNDE	2.275126	0.000994
205534_at	PCDH7	1.808023	0.002397	228962_at	PDE4D	2.273621	0.002435
207144_s_at	CITED1	1.525835	0.003498	212768_s_at	OLFM4	2.259513	0.000416
241368_at	PLIN5	1.471091	0.002259	205214_at	STK17B	2.178595	0.003404
1555236_a_at	PGC	−3.97182	0.000378	223761_at	FGF19	−4.87686	0.000303
219934_s_at	SULT1E1	−3.25992	0.002483	207016_s_at	ALDH1A2	−4.52882	0.004594
223509_at	CLDN2	−2.84658	0.000757	219106_s_at	KBTBD10	−4.32228	0.001409
226960_at	CXCL17	−2.76824	0.001549	209277_at	TFPI2	−4.17033	0.000529
201236_s_at	BTG2	−2.4767	0.005068	209993_at	ABCB1	−4.04187	0.000584
228912_at	VIL1	−2.40512	0.001675	204965_at	GC	−3.8697	0.003231
229254_at	MFSD4	−2.23402	0.00422	234673_at	HHLA2	−3.84065	3.20E−05
243296_at	NAMPT	−2.20159	0.000229	222257_s_at	ACE2	−3.54203	0.000581
1562625_at	FRYL	−2.08383	0.005056	205380_at	PDZK1	−3.37185	0.000588
225283_at	ARRDC4	−1.95739	0.003397	214397_at	MBD2	−3.23842	0.004437
Probe Set ID (Individual P3)	**Gene Symbol**	**Fold Change**	***P***	**Probe Set ID (Individual P4)**	**Gene Symbol**	**Fold Change**	***P***
204920_at	CPS1	4.650319	0.000671	215867_x_at	CA12	2.933468	0.00025
239196_at	ANKRD22	4.62406	0.000438	208268_at	ADAM28	2.429627	0.004945
206291_at	NTS	4.048737	0.003113	235155_at	BDH2	1.938958	0.001707
220639_at	TM4SF20	3.977558	0.000243	229241_at	LDHD	1.735299	0.002328
218173_s_at	WHSC1L1	3.895598	1.04E−05	206242_at	TM4SF5	1.611106	0.000131
209806_at	HIST1H2BK	3.831179	0.003936	204602_at	DKK1	1.53691	0.003798
230252_at	LPAR5	3.741123	0.001988	224224_s_at	PDE11A	1.501315	0.001659
40020_at	CELSR3	3.695498	0.002774	1567079_at	CLN6	1.498309	0.00314
1557129_a_at	FAM111B	3.592115	0.000226	236129_at	GALNT5	1.434861	0.004932
1556357_s_at	ERICH1	3.544031	0.002244	219404_at	EPS8L3	1.428246	0.00397
214411_x_at	CTRB2	−5.7882	0.000797	219179_at	DACT1	−3.6374	0.001905
211766_s_at	PNLIPRP2	−5.52339	0.001025	213680_at	KRT6B	−3.3096	0.00037
207802_at	CRISP3	−5.49445	0.00027	206227_at	CILP	−2.94772	0.000918
205971_s_at	CTRB1///CTRB2	−5.42292	0.000386	204464_s_at	EDNRA	−2.86192	0.003475
205886_at	REG1B	−5.25456	0.000635	1560224_at	AHCTF1	−2.56906	0.000756
205509_at	CPB1	−5.24977	0.000438	226412_at	SFRS18	−2.51134	0.00462
209277_at	TFPI2	−4.04264	0.005253	225571_at	LIFR	−2.43013	0.005276
209616_s_at	CES1	−3.88049	0.003005	201108_s_at	THBS1	−2.42659	0.003524
207254_at	SLC15A1	−3.77115	0.000382	201838_s_at	SUPT7L	−2.42213	0.001748
211738_x_at	CELA3A	−3.54389	0.004419	1563321_s_at	MLLT10	−2.37822	0.004121

models.[28] Interleukin 12 is currently being investigated as a potential therapeutic agent against cancer.[29]

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.[30] 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.[31,32] 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, *CDK1* (cyclin-dependent kinase 1) that promotes phosphorylation of the proapoptotic *BAD* (BCL-2–associated agonist of cell death) was up-regulated in the BAD phosphorylation pathway. This is evidence for deregulation of the apoptosis and survival pathways in P4.

TABLE 6. Selective Genes From Table 5 Implicated in Pancreatic and Other Cancers

	Gene Symbol/ Gene Description	Fold Change*	Significance to Pancreatic Cancer and Other Cancers	Ref†
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/progastricsin	−4.43	Reduced expression associated with stomach cancer	68,69
	SULT1E1/sulfotransferase family 1E, estrogen-preferring, 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	2.73	Biomarker for breast cancer	78
	CD55/CD55 molecule, decay accelerating factor for complement (Cromer blood group)	2.63	Overexpression associated with breast and prostate cancer	79–81
	OLFM4/olfactomedin 4	2.26	Promotes proliferation of pancreatic cancer cells	82
	ALDH1A2/aldehyde dehydrogenase 1 family, member A2	−4.53	Tumor suppressor in prostate cancer	83
	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
	PNLIPRP2/pancreatic lipase–related protein 2	−5.52	Overexpression associated with poor prognosis in pancreatic cancer	93
	CTRB1/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/carbonic anhydrase XII	−2.93	Promotes tumor growth and invasion	95
	ADAM28/ADAM metallopeptidase domain 28	−2.43	Overexpressed in many malignant tumors	40,96
	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.

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 7. The Significantly ($P \leq 0.05$) 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 G_1/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—innate 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	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 adhesion—plasmin signaling	0.03519
		Cell cycle—regulation of G_1/S transition (part 1)	0.04091
		Cell adhesion—chemokines and adhesion	0.05265

P3 Pathway Maps	P	P4 Pathway Maps	P
Cell cycle—chromosome condensation in prometaphase	4.77E−06	Development—WNT signaling pathway. Part 2	0.00002776
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-nitrosoglutathione (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 G_2/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

(Continued on next page)

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 G_2/M checkpoint	0.04124	Estradiol metabolism/rodent version	0.02617
Glutathione metabolism/human version	0.04722	Cell cycle—regulation of G_1/S transition (part 1)	0.0275
Glycolysis and gluconeogenesis (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
		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

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
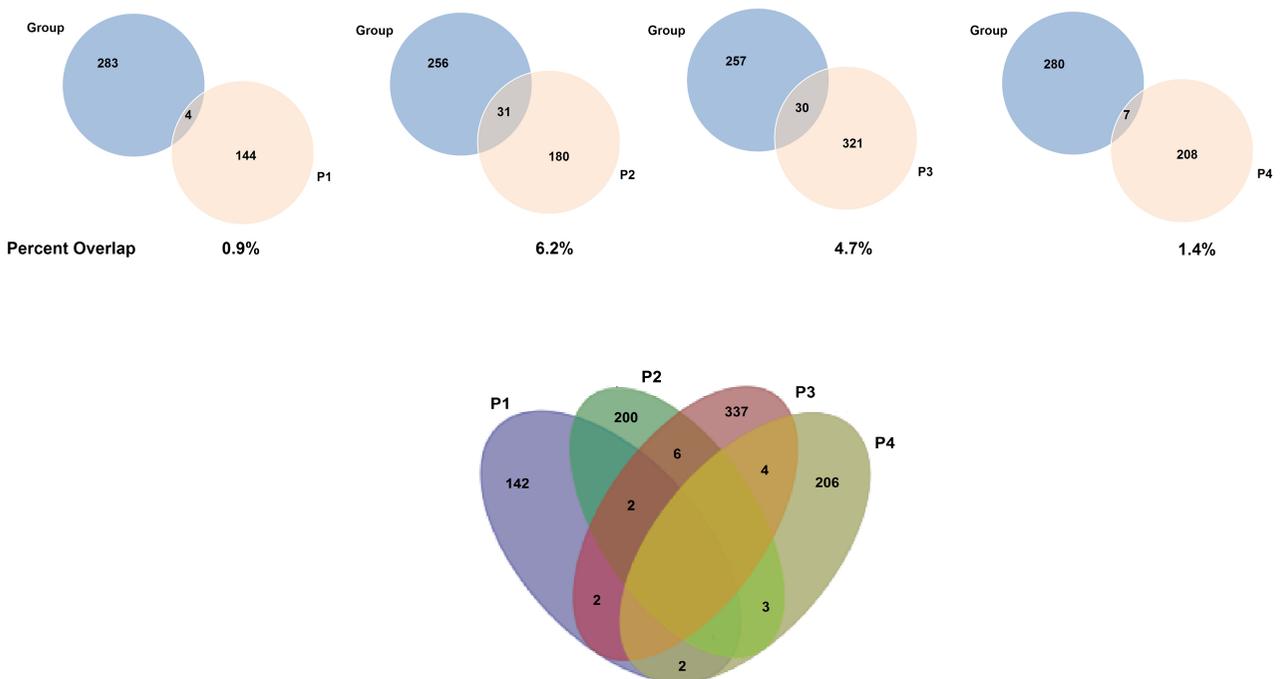

FIGURE 3. Venn diagrams showing the unique, annotated genes identified as significantly differentially expressed in the group analysis and in the personalized analysis(es) of at least 1 patient.
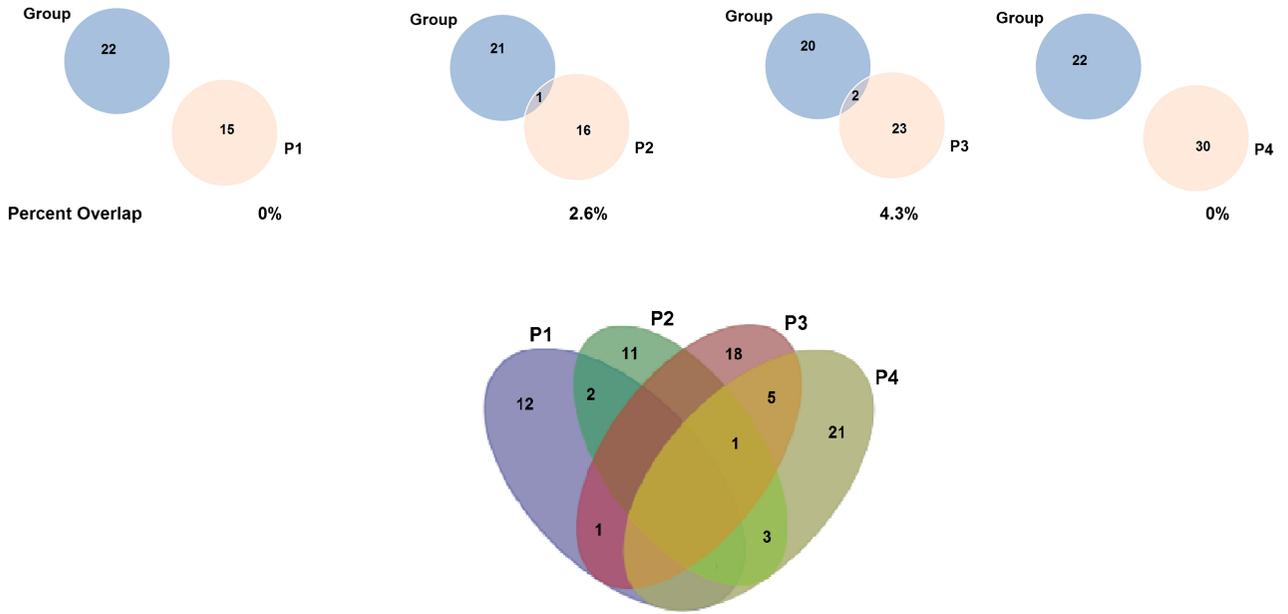
FIGURE 4. Venn diagrams showing pathways identified as significantly enriched in the group analysis and in the personalized analysis(es) of at least 1 patient.

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
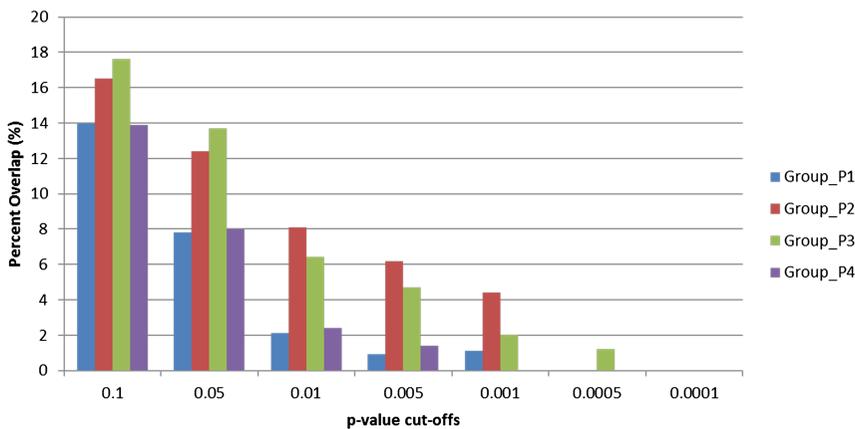

FIGURE 5. Histogram representing the percent overlap of differentially expressed genes found by group versus P1, group versus P2, group versus P3, and group versus P4 over a range of *P* values. Note that even at a nonsignificant cutoff *P* value of 0.1, the group versus personalized of P3 shows relatively little overlap (~18%).
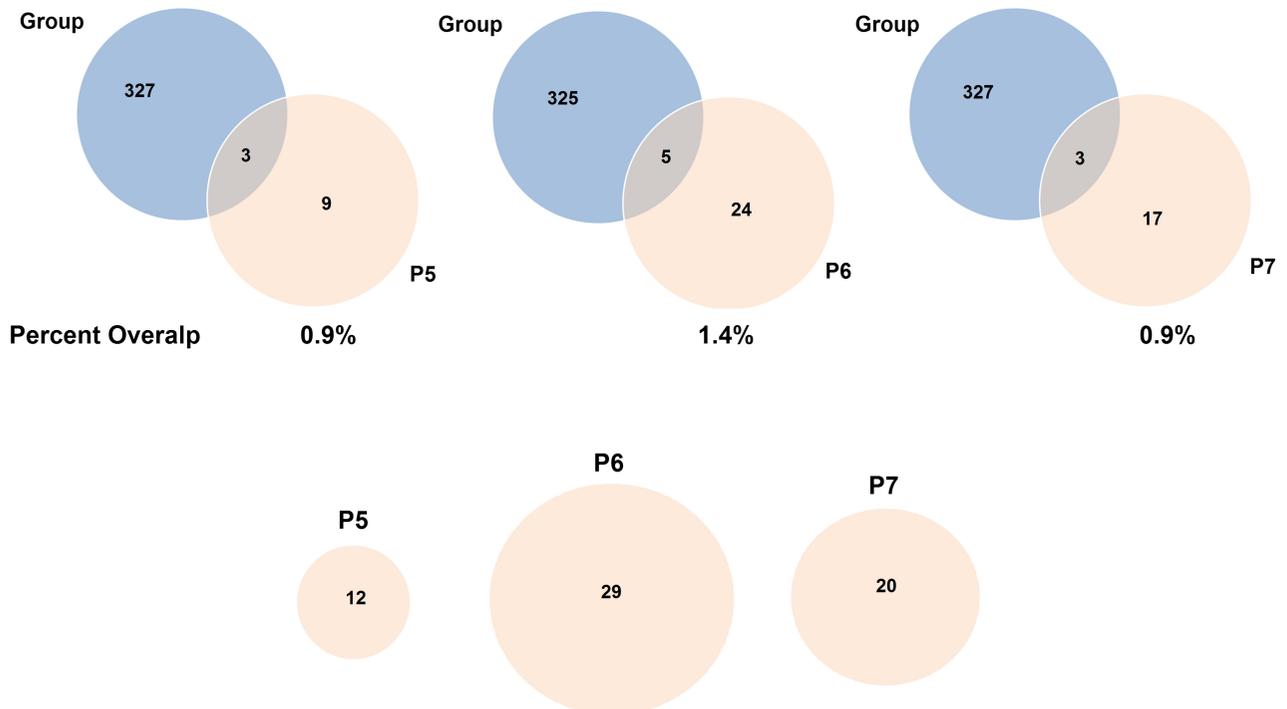
FIGURE 6. Venn diagrams showing the genes identified as significantly differentially expressed in the group analysis and in the personalized analysis(es) of at least 1 patient using data from Badea et al.[16]

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 ($P \leq 0.00001$) 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[33]). 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.[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,[13,15] suggesting that pancreatic cancer cannot be characterized by a narrowly defined set of mutations across all patients.[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[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,[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 both prostate and pancreatic cancer.[38,39] Thus, whereas PSCA targeted therapy might well be expected to be effective for P1, it was not identified as being significantly overexpressed in the group analysis or in the personalized analyses of any of the other patients examined. Similarly, *ADAM* (*a d*isintegrin *a*nd *m*etalloprotease), a gene reported to be overexpressed in a number of human cancers[40] and identified as a potential candidate for targeted gene therapy,[41] was among the most significantly overexpressed genes in P4 but was not identified as being significantly overexpressed in the group analysis or in the personalized analyses of any of the other patients examined.

Collectively, our results are consistent with earlier findings indicating that, on the molecular level, pancreatic cancer is a highly heterogeneous disease.[13] Although targeted gene therapy is believed by many to hold great promise in the treatment of pancreatic and other cancers, a crucial step in the process is the accurate identification of appropriate candidate genes for targeted therapy. Our findings indicate that personalized and not group molecular profiling is the most appropriate approach for the identification of putative candidates for effective targeted gene therapy for pancreatic and perhaps other cancers with heterogeneous molecular etiology.

REFERENCES

1. Idris SF, Ahmad SS, Scott MA, et al. The role of high-throughput technologies in clinical cancer genomics. *Expert Rev Mol Diagn*. 2013;13:167–181.
2. Lakhani SR, Ashworth A. Microarray and histopathological analysis of tumours: the future and the past? *Nat Rev Cancer*. 2001;1:151–157.
3. ten Bosch JR, Grody WW. Keeping up with the next generation: massively parallel sequencing in clinical diagnostics. *J Mol Diagn*. 2008;10:484–492.
4. Su Z, Ning B, Fang H, et al. Next-generation sequencing and its applications in molecular diagnostics. *Expert Rev Mol Diagn*. 2011;11:333–343.
5. Sotiriou C, Neo SY, McShane LM, et al. Breast cancer classification and prognosis based on gene expression profiles from a population-based study. *Proc Natl Acad Sci U S A*. 2003;100:10393–10398.
6. Chuang HY, Lee E, Liu YT, et al. Network-based classification of breast cancer metastasis. *Mol Syst Biol*. 2007;3:140.
7. Meyerson M, Gabriel S, Getz G. Advances in understanding cancer genomes through second-generation sequencing. *Nat Rev Genet*. 2010;11:685–696.
8. van't Veer LJ, Bernards R. Enabling personalized cancer medicine through analysis of gene-expression patterns. *Nature*. 2008;452:564–570.
9. Wulfkuhle J, Espina V, Liotta L, et al. Genomic and proteomic technologies for individualisation and improvement of cancer treatment. *Eur J Cancer*. 2004;40:2623–2632.
10. Altshuler D, Daly MJ, Lander ES. Genetic mapping in human disease. *Science*. 2008;322:881–888.
11. McClellan J, King MC. Genetic heterogeneity in human disease. *Cell*. 2010;141:210–217.
12. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. *Nat Med*. 2004;10:789–799.
13. Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science*. 2008;321:1801–1806.
14. Liang WS, Craig DW, Carpten J, et al. Genome-wide characterization of pancreatic adenocarcinoma patients using next generation sequencing. *PLoS One*. 2012;7:e43192.
15. Biankin AV, Waddell N, Kassahn KS, et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. *Nature*. 2012;491:399–405.
16. Badea L, Herlea V, Dima SO, et al. Combined gene expression analysis of whole-tissue and microdissected pancreatic ductal adenocarcinoma identifies genes specifically overexpressed in tumor epithelia. *Hepatogastroenterology*. 2008;55:2016–2027.
17. Grant SL, Begley CG. The oncostatin M signalling pathway: reversing the neoplastic phenotype? *Mol Med Today*. 1999;5:406–412.
18. Fernandez-del Castillo C, Robles-Diaz G, Diaz-Sanchez V, et al. Pancreatic cancer and androgen metabolism: high androstenedione and low testosterone serum levels. *Pancreas*. 1990;5:515–518.
19. Terabe M, Park JM, Berzofsky JA. Role of IL-13 in regulation of anti-tumor immunity and tumor growth. *Cancer Immunol Immunother*. 2004;53:79–85.
20. Murugaiyan G, Saha B. Protumor vs antitumor functions of IL-17. *J Immunol*. 2009;183:4169–4175.
21. Conti I, Rollins BJ. CCL2 (monocyte chemoattractant protein-1) and cancer. *Semin Cancer Biol*. 2004;14:149–154.
22. Muller MR, Rao A. NFAT, immunity and cancer: a transcription factor comes of age. *Nat Rev Immunol*. 2010;10:645–656.
23. Arrick BA, Nathan CF. Glutathione metabolism as a determinant of therapeutic efficacy: a review. *Cancer Res*. 1984;44:4224–4232.
24. Bolos V, Grego-Bessa J, de la Pompa JL. Notch signaling in development and cancer. *Endocr Rev*. 2007;28:339–363.
25. Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. *Nature*. 2004;432:316–323.
26. Yilmaz M, Christofori G. EMT, the cytoskeleton, and cancer cell invasion. *Cancer Metastasis Rev*. 2009;28:15–33.
27. Hirohashi S, Kanai Y. Cell adhesion system and human cancer morphogenesis. *Cancer Sci*. 2003;94:575–581.
28. Tahara H, Lotze MT. Antitumor effects of interleukin-12 (IL-12): applications for the immunotherapy and gene therapy of cancer. *Gene Ther*. 1995;2:96–106.
29. Colombo MP, Trinchieri G. Interleukin-12 in anti-tumor immunity and immunotherapy. *Cytokine Growth Factor Rev*. 2002;13:155–168.
30. Reya T, Clevers H. Wnt signalling in stem cells and cancer. *Nature*. 2005;434:843–850.
31. Kirikoshi H, Sekihara H, Katoh M. Up-regulation of Frizzled-7 (FZD7) in human gastric cancer. *Int J Oncol*. 2001;19:111–115.
32. Ueno K, Hiura M, Suehiro Y, et al. Frizzled-7 as a potential therapeutic target in colorectal cancer. *Neoplasia*. 2008;10:697–705.
33. Clarke GM, Anderson CA, Pettersson FH, et al. Basic statistical analysis in genetic case-control studies. *Nat Protoc*. 2011;6:121–133.
34. Williams SM, Canter JA, Crawford DC, et al. Problems with genome-wide association studies. *Science*. 2007;316:1840–1842.
35. Makohon-Moore A, Brosnan JA, Iacobuzio-Donahue CA. Pancreatic cancer genomics: insights and opportunities for clinical translation. *Genome Med*. 2013;5:26.
36. Andrianifahanana M, Moniaux N, Schmied BM, et al. Mucin (MUC) gene expression in human pancreatic adenocarcinoma and chronic pancreatitis: a potential role of MUC4 as a tumor marker of diagnostic significance. *Clin Cancer Res*. 2001;7:4033–4040.
37. Singh AP, Chaturvedi P, Batra SK. Emerging roles of MUC4 in cancer: a novel target for diagnosis and therapy. *Cancer Res*. 2007;67:433–436.
38. Antonarakis ES, Carducci MA, Eisenberger MA, et al. Phase I rapid dose-escalation study of AGS-1C4D4, a human anti-PSCA (prostate

stem cell antigen) monoclonal antibody, in patients with castration-resistant prostate cancer: a PCCTC trial. *Cancer Chemother Pharmacol*. 2012;69:763–771.

39. Wolpin BM, O'Relly EM, Ko Y. Global, multicenter, open-label, randomized phase II trial comparing gemcitabine (G) with G plus AGS-1C4D4 (A) in patients (pts) with metastatic pancreatic cancer (mPC). *J Clin Oncol*. 2011;29(suppl). Abstract 4031.

40. Mochizuki S, Okada Y. ADAMs in cancer cell proliferation and progression. *Cancer Sci*. 2007;98:621–628.

41. Lu X, Lu D, Scully M, et al. ADAM proteins—therapeutic potential in cancer. *Curr Cancer Drug Targets*. 2008;8:720–732.

42. Arumugam T, Simeone DM, van Golen K, et al. S100P promotes pancreatic cancer growth, survival, and invasion. *Clin Cancer Res*. 2005;11:5356–5364.

43. Basu GD, Azorsa DO, Kiefer JA, et al. Functional evidence implicating S100P in prostate cancer progression. *Int J Cancer*. 2008;123:330–339.

44. Baine MJ, Chakraborty S, Smith LM, et al. Transcriptional profiling of peripheral blood mononuclear cells in pancreatic cancer patients identifies novel genes with potential diagnostic utility. *PLoS One*. 2011;6:e17014.

45. Chaturvedi P, Singh AP, Moniaux N, et al. MUC4 mucin potentiates pancreatic tumor cell proliferation, survival, and invasive properties and interferes with its interaction to extracellular matrix proteins. *Mol Cancer Res*. 2007;5:309–320.

46. Chaturvedi P, Singh AP, Chakraborty S, et al. MUC4 mucin interacts with and stabilizes the HER2 oncoprotein in human pancreatic cancer cells. *Cancer Res*. 2008;68:2065–2070.

47. Graham LD, Pedersen SK, Brown GS, et al. Colorectal neoplasia differentially expressed (CRNDE), a novel gene with elevated expression in colorectal adenomas and adenocarcinomas. *Genes Cancer*. 2011;2:829–840.

48. Chae YK, Kang SK, Kim MS, et al. Human AQP5 plays a role in the progression of chronic myelogenous leukemia (CML). *PLoS One*. 2008;3:e2594.

49. Chae YK, Woo J, Kim MJ, et al. Expression of aquaporin 5 (AQP5) promotes tumor invasion in human non small cell lung cancer. *PLoS One*. 2008;3:e2162.

50. Jung HJ, Park JY, Jeon HS, et al. Aquaporin-5: a marker protein for proliferation and migration of human breast cancer cells. *PLoS One*. 2011;6:e28492.

51. Kang SK, Chae YK, Woo J, et al. Role of human aquaporin 5 in colorectal carcinogenesis. *Am J Pathol*. 2008;173:518–525.

52. Woo J, Lee J, Chae YK, et al. Overexpression of AQP5, a putative oncogene, promotes cell growth and transformation. *Cancer Lett*. 2008;264:54–62.

53. Zhang Z, Chen Z, Song Y, et al. Expression of aquaporin 5 increases proliferation and metastasis potential of lung cancer. *J Pathol*. 2010;221:210–220.

54. Chand HS, Foster DC, Kisiel W. Structure, function and biology of tissue factor pathway inhibitor-2. *Thromb Haemost*. 2005;94:1122–1130.

55. Glockner SC, Dhir M, Yi JM, et al. Methylation of TFPI2 in stool DNA: a potential novel biomarker for the detection of colorectal cancer. *Cancer Res*. 2009;69:4691–4699.

56. Sato N, Parker AR, Fukushima N, et al. Epigenetic inactivation of TFPI-2 as a common mechanism associated with growth and invasion of pancreatic ductal adenocarcinoma. *Oncogene*. 2005;24:850–858.

57. Sierko E, Wojtukiewicz MZ, Kisiel W. The role of tissue factor pathway inhibitor-2 in cancer biology. *Semin Thromb Hemost*. 2007;33:653–659.

58. Turner N, Grose R. Fibroblast growth factor signalling: from development to cancer. *Nat Rev Cancer*. 2010;10:116–129.

59. Lauffart B, Vaughan MM, Eddy R, et al. Aberrations of TACC1 and TACC3 are associated with ovarian cancer. *BMC Womens Health*. 2005;5:8.

60. Argani P, Rosty C, Reiter RE, et al. Discovery of new markers of cancer through serial analysis of gene expression: prostate stem cell antigen is overexpressed in pancreatic adenocarcinoma. *Cancer Res*. 2001;61:4320–4324.

61. He P, Varticovski L, Bowman ED, et al. Identification of carboxypeptidase E and gamma-glutamyl hydrolase as biomarkers for pulmonary neuroendocrine tumors by cDNA microarray. *Hum Pathol*. 2004;35:1196–1209.

62. Karlsson E, Delle 2U, Danielsson A, et al. Gene expression variation to predict 10-year survival in lymph-node–negative breast cancer. *BMC Cancer*. 2008;8:254.

63. van Houdt IS, Oudejans JJ, van den Eertwegh AJ, et al. Expression of the apoptosis inhibitor protease inhibitor 9 predicts clinical outcome in vaccinated patients with stage III and IV melanoma. *Clin Cancer Res*. 2005;11:6400–6407.

64. Yoshikawa R, Yanagi H, Shen CS, et al. ECA39 is a novel distant metastasis-related biomarker in colorectal cancer. *World J Gastroenterol*. 2006;12:5884–5889.

65. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer*. 2002;2:161–174.

66. Foda HD, Zucker S. Matrix metalloproteinases in cancer invasion, metastasis and angiogenesis. *Drug Discov Today*. 2001;6:478–482.

67. Peruzzi D, Mori F, Conforti A, et al. MMP11: a novel target antigen for cancer immunotherapy. *Clin Cancer Res*. 2009;15:4104–4113.

68. Hasegawa S, Furukawa Y, Li M, et al. Genome-wide analysis of gene expression in intestinal-type gastric cancers using a complementary DNA microarray representing 23,040 genes. *Cancer Res*. 2002;62:7012–7017.

69. Kim B, Bang S, Lee S, et al. Expression profiling and subtype-specific expression of stomach cancer. *Cancer Res*. 2003;63:8248–8255.

70. Falany CN, Meloche CA, He D, et al. Expression and subcellular localization of human sulfotransferases (SULTs) in normal and cancerous prostate and breast tissues. *Proc Am Assoc Cancer Res*. 2004;2004:1020.

71. Hewitt KJ, Agarwal R, Morin PJ. The claudin gene family: expression in normal and neoplastic tissues. *BMC Cancer*. 2006;6:186.

72. Hiraoka N, Yamazaki-Itoh R, Ino Y, et al. CXCL17 and ICAM2 are associated with a potential anti-tumor immune response in early intraepithelial stages of human pancreatic carcinogenesis. *Gastroenterology*. 2011;140:310–321.

73. Kawakubo H, Carey JL, Brachtel E, et al. Expression of the NF-kappaB–responsive gene *BTG2* is aberrantly regulated in breast cancer. *Oncogene*. 2004;23:8310–8319.

74. Rouault JP, Falette N, Guehenneux F, et al. Identification of BTG2, an antiproliferative p53-dependent component of the DNA damage cellular response pathway. *Nat Genet*. 1996;14:482–486.

75. Arango D, Al-Obaidi S, Williams DS, et al. Villin expression is frequently lost in poorly differentiated colon cancer. *Am J Pathol*. 2012;180:1509–1521.

76. Galli M, van Gool F, Rongvaux A, et al. The nicotinamide phosphoribosyltransferase: a molecular link between metabolism, inflammation, and cancer. *Cancer Res*. 2010;70:8–11.

77. Garten A, Petzold S, Korner A, et al. Nampt: linking NAD biology, metabolism and cancer. *Trends Endocrinol Metab*. 2009;20:130–138.

78. Siddiqui S, Bruker CT, Kestler DP, et al. Odontogenic ameloblast associated protein as a novel biomarker for human breast cancer. *Am Surg*. 2009;75:769–775; discussion 775.

79. Bjorge L, Hakulinen J, Wahlstrom T, et al. Complement-regulatory proteins in ovarian malignancies. *Int J Cancer*. 1997;70:14–25.

80. Loberg RD, Day LL, Dunn R, et al. Inhibition of decay-accelerating factor (CD55) attenuates prostate cancer growth and survival in vivo. *Neoplasia*. 2006;8:69–78.

81. Rushmere NK, Knowlden JM, Gee JM, et al. Analysis of the level of mRNA expression of the membrane regulators of complement, CD59, CD55 and CD46, in breast cancer. *Int J Cancer*. 2003;108:930–936.

82. Kobayashi D, Koshida S, Moriai R, et al. Olfactomedin 4 promotes S-phase transition in proliferation of pancreatic cancer cells. *Cancer Sci*. 2007;98:334–340.

83. Kim H, Lapointe J, Kaygusuz G, et al. The retinoic acid synthesis gene *ALDH1a2* is a candidate tumor suppressor in prostate cancer. *Cancer Res*. 2005;65:8118–8124.

84. Zhou L, Zhang R, Zhang L, et al. Angiotensin-converting enzyme 2 acts as a potential molecular target for pancreatic cancer therapy. *Cancer Lett*. 2011;307:18–25.

85. Glavinas H, Krajcsi P, Cserepes J, et al. The role of ABC transporters in drug resistance, metabolism and toxicity. *Curr Drug Deliv*. 2004;1:27–42.

86. Ishizuka J, Townsend CM Jr, Thompson JC. Neurotensin regulates growth of human pancreatic cancer. *Ann Surg*. 1993;217:439–445; discussion 446.

87. Ryder NM, Guha S, Hines OJ, et al. G protein–coupled receptor signaling in human ductal pancreatic cancer cells: neurotensin responsiveness and mitogenic stimulation. *J Cell Physiol*. 2001;186:53–64.

88. Bernard-Pierrot I, Gruel N, Stransky N, et al. Characterization of the recurrent 8p11–12 amplicon identifies PPAPDC1B, a phosphatase protein, as a new therapeutic target in breast cancer. *Cancer Res*. 2008;68:7165–7175.

89. Kang D, Cho HS, Toyokawa G, et al. The histone methyltransferase Wolf-Hirschhorn syndrome candidate 1-like 1 (WHSC1L1) is involved in human carcinogenesis. *Genes Chromosomes Cancer*. 2013;52:126–139.

90. Erkan M, Hausmann S, Michalski CW, et al. The role of stroma in pancreatic cancer: diagnostic and therapeutic implications. *Nat Rev Gastroenterol Hepatol*. 2012;9:454–467.

91. Erkan M, Weis N, Pan Z, et al. Organ-, inflammation- and cancer specific transcriptional fingerprints of pancreatic and hepatic stellate cells. *Mol Cancer*. 2010;9:88.

92. Harada T, Chelala C, Crnogorac-Jurcevic T, et al. Genome-wide analysis of pancreatic cancer using microarray-based techniques. *Pancreatology*. 2009;9:13–24.

93. van den Broeck A, Vankelecom H, van Eijsden R, et al. Molecular markers associated with outcome and metastasis in human pancreatic cancer. *J Exp Clin Cancer Res*. 2012;31:68.

94. Grutzmann R, Foerder M, Alldinger I, et al. Gene expression profiles of microdissected pancreatic ductal adenocarcinoma. *Virchows Arch*. 2003;443:508–517.

95. Ivanov S, Liao SY, Ivanova A, et al. Expression of hypoxia-inducible cell-surface transmembrane carbonic anhydrases in human cancer. *Am J Pathol*. 2001;158:905–919.

96. Rocks N, Paulissen G, El Hour M, et al. Emerging roles of ADAM and ADAMTS metalloproteinases in cancer. *Biochimie*. 2008;90:369–379.

97. Yuan G, Wang C, Ma C, et al. Oncogenic function of DACT1 in colon cancer through the regulation of beta-catenin. *PLoS One*. 2012;7:e34004.

98. Lehmann BD, Bauer JA, Chen X, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest*. 2011;121:2750–2767.

99. Martoglio AM, Tom BD, Starkey M, et al. Changes in tumorigenesis- and angiogenesis-related gene transcript abundance profiles in ovarian cancer detected by tailored high density cDNA arrays. *Mol Med*. 2000;6:750–765.

100. Wang Y, Chen J, Li Q, et al. Identifying novel prostate cancer associated pathways based on integrative microarray data analysis. *Comput Biol Chem*. 2011;35:151–158.

101. Wiesmann F, Veeck J, Galm O, et al. Frequent loss of endothelin-3 (EDN3) expression due to epigenetic inactivation in human breast cancer. *Breast Cancer Res*. 2009;11:R34.