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# High-Resolution Genomic and Expression Profiling Reveals 105 Putative Amplification Target Genes in Pancreatic Cancer<sup>1</sup>

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## Abstract

Comparative genomic hybridization (CGH) studies have provided a wealth of information on common copy number aberrations in pancreatic cancer, but the genes affected by these aberrations are largely unknown. To identify putative amplification target genes in pancreatic cancer, we performed a parallel copy number and expression survey in 13 pancreatic cancer cell lines using a 12,232-clone cDNA microarray, providing an average resolution of 300 kb throughout the human genome. CGH on cDNA microarray allowed highly accurate mapping of copy number increases and resulted in identification of 24 independent amplicons, ranging in size from 130 kb to 11 Mb. Statistical evaluation of gene copy number and expression data across all 13 cell lines revealed a set of 105 genes whose elevated expression levels were directly attributable to increased copy number. These included genes previously reported to be amplified in cancer as well as several novel targets for copy number alterations, such as *p21-activated kinase 4 (PAK4)*, which was previously shown to be involved in cell migration, cell adhesion, and anchorage-independent growth. In conclusion, our results implicate a set of 105 genes that is likely to be actively involved in the development and progression of pancreatic cancer.

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**Keywords:** Pancreatic cancer, amplification, CGH microarray, cDNA microarray, overexpression.

## Introduction

Nearly 30,000 new pancreatic cancer cases were diagnosed in the United States in 2001, representing about 2.3% of all new cancer cases [1]. Despite this relatively low incidence, pancreatic cancer was the fourth common cause of cancer deaths in the United States, with 5-year survival rates of 3% to 5% [1,2]. The poor prognosis of pancreatic cancer is largely due to the fact that the first symptoms (e.g., jaundice caused by the growing tumor obstructing the common bile duct, or abdominal and back pain caused by perineural invasion in the celiac plexus)

come typically rather late in the disease progression. Therefore, most pancreatic cancers have already metastasized and are inoperable at the time of diagnosis [3,4].

There are several well-documented risk factors for the development of pancreatic cancer, including smoking [5], chronic pancreatitis [6], and a family history of pancreatic cancer [7,8]. However, the genetic changes involved in the pathogenesis of pancreatic cancer are not fully understood, although the role of a few known oncogenes and tumor-suppressor genes has been well established (reviewed in Ref. [9]). Mutations leading to activation of the *KRAS* oncogene are the most common genetic changes in pancreatic cancer occurring in nearly all primary tumors [9,10]. *KRAS* mutations have been observed already in normal pancreas as well as in noninvasive neoplastic precursor lesions, indicating that they represent an early event in the pathogenesis of pancreatic cancer [11]. Furthermore, overexpression of *EGFR* and *MYC*, as well as inactivation of *TP53* and *MADH4*, have been frequently detected in pancreatic cancer [10,12–14]. In addition to these alterations involving known oncogenes or tumor-suppressor genes, cytogenetic and molecular cytogenetic studies have revealed frequent structural and numeric chromosome abnormalities in pancreatic cancer. For example, comparative genomic hybridization (CGH) analyses have indicated common gains affecting chromosomes 7, 8q, 17q, 19q, and 20q, and losses of 6q, 8p, 9p, and 18q in pancreatic adenocarcinomas [15–19].

Recently, DNA microarray-based methods have been applied to the analysis of pancreatic cancer, mainly in an effort to identify new diagnostic markers or targets for the development of new therapies against pancreatic cancer [20–25]. These studies have revealed a considerable number of differentially expressed genes, the majority of which has not been previously

Abbreviations: CGH, comparative genomic hybridization

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implicated in pancreatic cancer. For example, Iacobuzio-Donahue et al. [24] identified a set of more than 400 genes that were differentially expressed in pancreatic cancer tissues and cell lines as compared to normal pancreas. These genes were linked to multiple cellular processes, such as cell–cell and cell–matrix interactions, cytoskeletal remodeling, proteolytic activity, and  $\text{Ca}^{2+}$  homeostasis. A set of 149 genes was more highly expressed in pancreatic cancers compared with normal pancreas and contained 103 genes that had not been previously reported in association with pancreatic cancer [24]. However, the exact role of these differentially expressed genes discovered by Iacobuzio-Donahue et al. [24] and others in pancreatic cancer pathogenesis remains to be elucidated.

In this study, we used a combination of CGH and cDNA microarray technologies to specifically identify those gene expression change events that were associated with gene copy number alterations. Analysis of 13 pancreatic cancer cell lines using a 12,232-clone cDNA microarray revealed 24 independent amplicons and a set of 105 genes whose expression levels were directly attributable to increased copy number. We hypothesize that these genes are likely to have a central role in the pathogenesis of pancreatic cancer.

## Materials and Methods

### Cell Lines

Thirteen established cell lines derived from pancreatic adenocarcinomas or their metastases (AsPC-1, BxPC-3, Capan-1, Capan-2, CFPAC-1, HPAC, HPAF-II, Hs 700T, Hs 766T, MIA PaCa-2, PANC-1, SU.86.86, and SW 1990) were obtained from the American Type Culture Collection (ATCC; Manassas, VA). The CFPAC-1 cell line originated from a patient with cystic fibrosis. The cells were grown under recommended culture conditions. Genomic DNA was isolated using standard protocols and mRNA was extracted with FastTrack 2.0 Kit (Invitrogen, Carlsbad, CA). Both DNA and mRNA preparations were derived from a single harvest of cells.

### cDNA Microarray–Based Copy Number and Expression Analyses

The overall experimental design consisted of two parallel microarray experiments where gene copy numbers and gene expression levels were measured in 13 pancreatic cancer cell lines by using an identical cDNA microarray. The cDNA microarray contained polymerase chain reaction (PCR)–amplified inserts derived from a total of 12,232 cDNA clones, representing 8881 different transcripts printed on poly-L-lysine–coated glass slides as previously described [26,27] (full description of the clones and their locations on the array is provided at [http://sigwww.cs.tut.fi/TICSP/Mahlamaki\\_et\\_al\\_2003/](http://sigwww.cs.tut.fi/TICSP/Mahlamaki_et_al_2003/)). All experiments were performed using slides from a single print set.

Copy number analyses were performed as previously reported [28,29]. Briefly, genomic DNA from cell lines and sex-matched normal human white blood cells were digested for 14 to 18 hours with *AluI* and *RsaI* restriction enzymes

(Life Technologies, Inc., Rockville, MD) and purified by phenol/chloroform extraction. Six micrograms of digested cell line and normal DNA was labeled with Cy3-dUTP and Cy5-dUTP (Amersham Pharmacia, Piscataway, NJ), respectively, using Bioprime Labeling kit (Life Technologies, Inc.). The hybridization mixture, containing the labeled probes, 150  $\mu\text{g}$  of *CoI* DNA (Life Technologies, Inc.), 300  $\mu\text{g}$  of yeast tRNA (Gibco/BRL, Gaithersburg, MD), as well as 60  $\mu\text{g}$  each of poly(dA) and poly(dT) in  $3.4 \times \text{SSC}/0.3\%$  sodium dodecyl sulfate (SDS), was denatured at  $100^\circ\text{C}$  for 1.5 minutes, incubated for 30 minutes at  $37^\circ\text{C}$ , and hybridized to a microarray slide at  $65^\circ\text{C}$  for 16 to 24 hours in a sealed humidified chamber. The slides were then washed in  $0.1\%$  SDS,  $0.5 \times \text{SSC}/0.01\%$  SDS, and  $0.06 \times \text{SSC}$  for 2 minutes each. For expression analyses, a pool of mRNA derived from all 13 cell lines was used as a common reference. Four micrograms of cell line mRNA was labeled with Cy3-dUTP and an equal amount of control mRNA was labeled with Cy5-dUTP by use of oligo(dT)-primed polymerization by SuperScript II reverse transcriptase (Life Technologies, Inc.). The labeled cDNA were hybridized on microarrays as described [27,29]. Briefly, they were combined with 12  $\mu\text{g}$  of poly(dA) (Pharmacia, Bridgewater, NJ), 6  $\mu\text{g}$  of tRNA, and 10  $\mu\text{g}$  of *CoI* DNA (Life Technologies, Inc.) in  $0.25\%$  SDS/ $2 \times \text{SSC}$ . The probe mix was incubated at  $98^\circ\text{C}$  for 2 minutes and at  $4^\circ\text{C}$  for 10 seconds, and hybridized on a cDNA microarray. Hybridization and subsequent washing were carried out as described above for copy number analyses.

For both copy number and expression analyses, a laser confocal scanner (Agilent Technologies, Palo Alto, CA) was used to measure the fluorescence intensities at the target locations. The fluorescent images from the test and control hybridizations were scanned separately, and image analysis was performed using the DeARRAY software [30]. After background subtraction, the average intensity at each clone in the test hybridization was divided by the average intensity of the corresponding clone in the control hybridization. Within-slide normalization for each cDNA and CGH microarray was performed using the Local Weighted Scatter Plot Smoother (LOWESS) method [31,32] for each print tip group. Fraction of data points used in local regression ( $f$ ) was 0.4 and other parameters were adjusted as suggested by Cleveland [32]. After within-slide normalization, low quality measurements (i.e., copy number data with mean reference intensity less than 50 fluorescent units, and expression data with both test and reference intensity less than 100 fluorescent units and/or with spot size less than 50 units) were excluded from the analysis and were treated as missing values. Self *versus* self experiments were performed to ensure the performance of copy number and expression hybridizations. In addition, 15 clones were printed as triplicates on the microarray. Evaluation of data derived from these 15 clones showed highly consistent results for each of the 13 cell lines with standard deviations ranging between 0.01 and 0.346 and between 0.011 and 0.427, for copy number and expression data, respectively (all original data are available at [http://sigwww.cs.tut.fi/TICSP/Mahlamaki\\_et\\_al\\_2003/](http://sigwww.cs.tut.fi/TICSP/Mahlamaki_et_al_2003/)).

### Identification of Amplicons

The chromosome and base-pair positions for each cDNA clone on the array were determined by using information from the November 2002 freeze of the University of California Santa Cruz's GoldenPath database ([www.genome.ucsc.edu](http://www.genome.ucsc.edu)) as described [33]. This information was available for 10,389 clones, providing an average spacing of 308 kb throughout the human genome. The CGH copy number data were ordered according to the location of the clones along chromosomes. Genes with copy number ratio >1.4 (representing the upper 5% of the CGH ratios across all experiments) were considered to be amplified. Two different criteria were used to define amplicons. First, based on the fact that typical amplicons span a large region of the genome, six (providing an average coverage of 1.5 Mb) or more adjacent clones were expected to show a copy number ratio >1.4. Secondly, to avoid missing small amplicons or amplicons located in regions of the genome with poorer-than-average clone coverage, regions with at least three adjacent clones with a copy number ratio >1.4 and no less than one clone with a ratio >2.0 were also considered as amplicons. The amplicon start and end positions were extended to include neighboring nonamplified clones (ratio <1.4). The amplicon size determination was dependent on the local clone density.

### Statistical Analyses

The influence of gene copy number on gene expression level was evaluated as described [33,34]. Briefly, within-slide normalized CGH and cDNA ratios in each cell line were log-transformed and median-centered. Furthermore, cDNA data were median-centered using values across 13 cell lines. For each gene, the CGH data were represented by a vector that was labeled "1" for amplification ratio >1.4 and "0" for no amplification. Amplification was correlated with gene expression using the signal-to-noise statistics [33,34]. A weight  $w_g$  was calculated for each gene:

$$w_g = \frac{m_{g1} - m_{g0}}{\sigma_{g1} + \sigma_{g0}}$$

where  $m_{g1}, \sigma_{g1}$  and  $m_{g0}, \sigma_{g0}$  denote the means and standard deviations for the expression levels for amplified and non-amplified cell lines, respectively. To assess the statistical significance of each weight, 10,000 random permutations of the label vector were performed. The probability that a gene had a larger or equal weight by random permutation than the original weight was denoted by  $\alpha$ . A low  $\alpha$  (<0.05) indicates a strong association between gene expression and amplification.

### Results

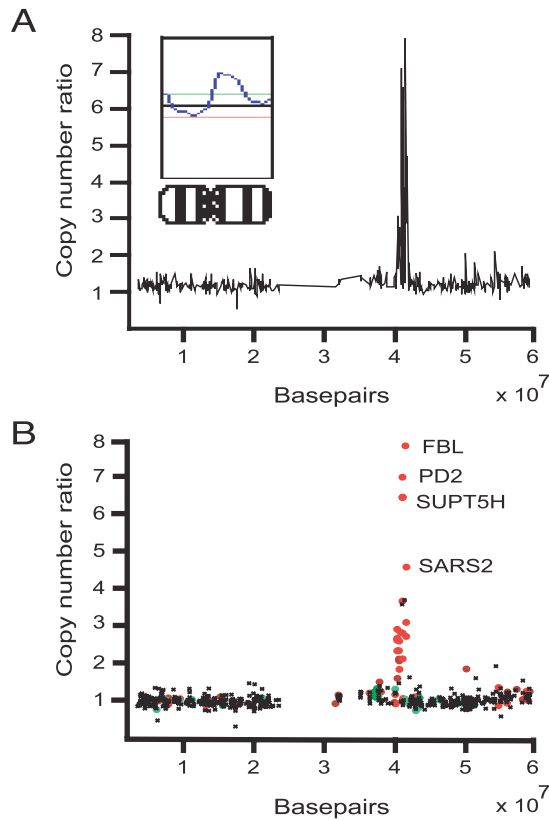
A genome-wide copy number analysis was performed in 13 pancreatic cancer cell lines using CGH on a cDNA microarray containing 12,232 clones. Chromosomal and base-pair locations were obtained for 10,389 clones, providing an average spacing of 308 kb throughout the human genome. The clone density varied considerably from one region of

the genome to another, with chromosome 19 showing the highest clone density (on average, one clone per 80 kb) and chromosome 13 having the poorest clone density (one clone per 1 Mb). Evaluation of copy number changes as a function of the genomic position of the clones revealed a total number of 24 independent amplicons in the 13 cell lines (Table 1). The number of amplicons varied from one cell line to another, with Capan1 cells showing the highest number of amplicons (8 of 24). The amplicons were located on 12 different chromosomes with multiple separate amplicons observed on chromosomes 15q, 17q, and 19. The use of base-pair information allowed exact determination of the locations and boundaries of each amplicon (Table 1). In several cases, the CGH microarray analysis resulted in high-resolution mapping of amplicons previously detected by chromosomal CGH (Figure 1A). The size of the amplicons ranged from 130 kb to 11 Mb, with an average of 2 Mb (Table 1), although it has to be noted that this size determination was dependent on the local clone density.

Next we analyzed the expression levels of the 12,232 cDNA clones in the 13 pancreatic cancer cell lines using an identical cDNA microarray. Direct comparison between copy number and expression levels in each cell line allowed unambiguous identification of genes whose expression levels were elevated due to increased copy number. For example, annotation of CGH copy number information with color-coded expression data revealed increased expression of several highly amplified genes, such as *FBL*, *PD2*, *SUPT5H*, and *SARS2*, located at the 19q13.1 region in the PANC-1 cell line (Figure 1B). Although such annotation is extremely effective in pinpointing putative amplification target genes in individual samples, it becomes progressively more complicated when the number of genes and samples to be analyzed increases. To facilitate the identification of

**Table 1.** Summary of Independent Amplicons in 13 Pancreatic Cancer Cell Lines by CGH on cDNA Microarray.

Location	Start (Mb)	End (Mb)	Size (Mb)
3p21	39.73	42.01	2.28
3q29	198.68	199.49	0.81
5p15.3	0.19	0.48	0.29
6p21	29.97	30.91	0.94
6p21	30.94	32.48	1.55
7q21	90.70	94.85	4.15
7q22	95.13	99.58	4.44
8q24	133.88	135.60	1.72
11q13	70.15	71.17	1.02
14q21	44.90	48.66	3.76
15q22	66.15	69.29	3.14
15q24	74.74	76.26	1.52
15q25	83.12	84.32	1.20
15q26	84.98	96.03	11.05
17q21	39.56	40.31	0.75
17q21	42.27	42.71	0.44
17q21	44.46	44.60	0.14
17q25	82.61	83.27	0.66
19p13.3	1.97	2.11	0.13
19q13.1	39.26	42.12	2.86
19q13.3	50.85	51.24	0.39
20q13.3	62.13	62.28	0.15
Xp22.3	2.72	11.76	9.05
Xq28	149.31	149.66	0.35



**Figure 1.** Gene copy number and expression analysis for chromosome 19 in the *Panc1* pancreatic cancer cell line by cDNA microarray. The copy number ratios were plotted as a function of the position of the cDNA clones along chromosome 19. In (A), individual data points were connected with a line. The inset shows chromosomal CGH copy number ratio profile. In (B), individual data points were color-coded according to cDNA expression ratios. The red dots indicate overexpressed genes (the upper 7% of expression ratios) and the green dots indicate underexpressed genes (lowest 7% of expression ratios) in *Panc1* cells.

possible amplification target genes, we applied a statistical approach with random permutation tests to explore the effects of gene copy number on gene expression levels across all 13 cell lines. This analysis revealed a set of 105 genes, including 70 known genes, whose expression levels were highly dependent on gene copy number (i.e., these genes were activated by increased copy number in the pancreatic cancer cell lines) (Table 2). The set included genes previously shown to be amplified in human cancer (e.g., *MLN51*) as well as known oncogenes (e.g., *RAB4A*, member of the *RAS* oncogene family). In addition, 11 (16%) of the 70 known genes (*ARHGDI3*, *CLDN4*, *FBL*, *MCM7*, *PSMC4*, *RELA*, *SIRT2*, *ST14*, *STK15*, *SUPT5H*, and *TRIAD3*) had been shown to be highly expressed in previous global gene expression surveys by either DNA microarrays [20–25] or serial analysis of gene expression (<http://www.ncbi.nlm.nih.gov/SAGE/>).

To obtain further information on the possible role of the 105 genes in the pathogenesis of pancreatic cancer, we explored their functional characteristics by using the SOURCE database (<http://source.stanford.edu>). According to the SOURCE, 17 of 105 genes represented hypothetical proteins and 21 were known genes or ESTs with no func-

tional annotation. A large fraction (78%) of the remaining 67 genes was involved in key cellular processes including signal transduction (17 genes), protein processing (11), metabolism (8), RNA processing (7), transcription (5), and DNA replication (4).

## Discussion

Pancreatic ductal adenocarcinoma is one of the top 10 causes of cancer death in industrialized countries, with over 40,000 deaths/year in Europe [35] and nearly 30,000 deaths/year in the United States [1]. New methods for early detection, a better understanding of the biologic mechanisms underlying cancer progression, and cancer-targeted treatment modalities are urgently needed to reduce the mortality from this lethal disease. The availability of global expression and copy number platforms, such as cDNA microarrays, has greatly facilitated the identification of novel tumor markers in many cancer types. Here we used a 12,232-clone cDNA microarray to search for genes that are activated by increased copy number in pancreatic cancer. The main focus of this study was to specifically identify genes whose overexpression is caused by increased copy number (i.e., those genes that are overexpressed only in cell lines with amplification). Gene copy numbers were first determined by performing CGH on the cDNA microarray, and subsequent expression analysis using an identical cDNA microarray permitted direct correlation between copy numbers and expression levels on a gene-by-gene basis.

The high-resolution copy number analysis by CGH microarray revealed a total of 24 independent regions of copy number increase in the 13 pancreatic cancer cell lines. These included several chromosomal segments, such as 3q, 5p, 7q, 8q24, 11q13, 15q, 17q, 19q, and 20q, previously implicated to be gained or amplified by chromosomal CGH or fluorescence *in situ* hybridization in pancreatic cancer [17,36,37]. In addition to validating data from these previous studies, the CGH microarray analysis permitted mapping of these copy number increases in much higher accuracy and determination of the exact base-pair boundaries for each aberration. For example, frequent copy number increases were observed at three separate regions on chromosome 19 (19p13.3, 19q13.1, and 19q13.3). Although gains and amplifications of 19q have been frequently reported both in pancreatic cancer cell lines and primary pancreatic carcinomas [17,18,36], the CGH microarray analysis was able to narrow down the affected regions to sizes of 130 kb, 2.9 Mb, and 390 kb, respectively (Table 1). However, it has to be noted that the size determination was fully dependent on the local clone density on the microarray and therefore does not necessarily correspond to the actual size of the amplicon.

The CGH microarray results obtained in this study considerably advance our knowledge on common copy number increases in pancreatic cancer and provide an improved starting point for the identification of genes affected by such copy number alterations. The subsequent expression survey performed using an identical cDNA microarray revealed that several genes located within the regions of increased copy

**Table 2.** List of 105 Genes with Statistically Significant Correlation ( $\alpha < 0.05$ ) between Gene Copy Number and Expression Level.

Gene Name	Clone ID	Locus	$\alpha$
EST	4541713		0.0035
CDC42-binding protein kinase beta (DMPK-like)	4582722	14q32.3	0.0045
POP7 (processing of precursor, <i>Saccharomyces cerevisiae</i> ) homolog	3138336	7q22	0.0047
TRIAD3 protein*	3506949	7p22.2	0.0073
Hypothetical protein F231491	3504227	19q13.1	0.0083
Hypothetical protein DKFZp434H247	3010753	19q13.13	0.0104
Hypothetical protein FLJ10055	37134	17q24	0.0107
FK506-binding protein 4 (59 kDa)	3940730	12p13.33	0.0109
Hypothetical protein MGC3234	3504261	11p15.5	0.0111
Opioid growth factor receptor	3050950	20q13.3	0.0112
Tubulin, gamma 1	108377	17q21	0.0117
Mitochondrial ribosomal protein S12	2959394	19q13.1-q13.2	0.0118
Ribosomal protein L8	3506015	8q24.3	0.012
ATP-dependent RNA helicase	3687438	17q21.1	0.0123
EST	3503397		0.0125
$\beta$ -1,3-Glucuronyltransferase 3 (glucuronosyltransferase I)	4299539	11q12.2	0.0127
EST	3543708		0.0129
p21 (CDKN1A)-activated kinase 4	3956856	19q13.1	0.0129
seryl-tRNA synthetase 2	3627808	19q13.13	0.0129
Metaxin 2	3542457	2q31.2	0.013
Sirtuin (silent mating type information regulation 2 homolog) 2*	2820929	19q13	0.013
Hypothetical protein FLJ20721	3544728	17q25.1	0.0132
Rho GDP dissociation inhibitor (GDI) $\beta$ -*	3621138	12p12.3	0.0133
Ring finger protein 26	3028875	11q23	0.0133
KIAA0182 protein	2819273	16q24	0.014
Ribosomal protein S16	4310307	19q13.1	0.0141
MCM2 minichromosome maintenance deficient 2	3544403	3q21	0.0145
Cyclin D-binding myb-like transcription factor 1	3010038	7q21	0.0146
Hypothetical gene <i>ZD52F10</i>	3690018	19q13.11	0.015
RNA-binding motif protein 10	3163064	Xp11.23	0.015
Cleavage and polyadenylation specific factor 4 (30 kDa)	3356071	7q22.1	0.0152
Fibrillarin*	3504198	19q13.1	0.0153
Hypothetical protein FLJ14888	3141437	15q25.1	0.0157
Hypothetical protein MGC3040	3163990	3q21	0.0157
Suppressor of Ty 5 homolog ( <i>S. cerevisiae</i> )*	3532191	19q13	0.0159
Succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	3051442	5p15	0.0164
EST	4543771		0.0167
suppression of tumorigenicity 14 (colon carcinoma)*	2959447	11q24-q25	0.0173
GTP-binding protein 1	4127961	22q13.1	0.0175
Nuclear factor I/C (CCAAT-binding transcription factor)	4128493	19p13.3	0.0183
Similar to RIKEN cDNA 0610011N22	4334552	5p15.33	0.0185
Similar to RIKEN cDNA <i>1810006A16</i> gene	3942185	16q22.1	0.0186
Syndecan 1	3347793	2p24.1	0.0187
ADP ribosylation factor GTPase-activating protein 1	3162991	20q13.33	0.0191
Likely ortholog of mouse another partner for ARF 1	2822464	14q24.3	0.0192
Proteasome (prosome, macropain) 26S subunit, ATPase, 4*	4046205	19q13.11-q13.13	0.0202
EST	3628799		0.0203
Hypothetical protein MGC45840	3941077	11p15.5	0.0205
PCTAIRE protein kinase 1	3504276	Xp11.3-p11.23	0.0205
EST	3958225		0.0209
Hypothetical protein BC011824	3830276	19p13.3	0.021
DKFZP564B147 protein	2821721	Xq26.3	0.0213
Claudin 4*	3349211	7q11.23	0.022
EST, weakly similar to IDN3 protein, isoform B	3528352		0.0221
Major histocompatibility complex, class I, C	4249847	6p21.3	0.0225
<i>Homo sapiens</i> , clone IMAGE: 3029191, mRNA	3029191	7	0.0228
Inhibitor of kappa light polypeptide gene enhancer in B-cells	4651870	Xq28	0.0233
EST	3678374		0.0238
Proteasome (prosome, macropain) subunit, $\alpha$ type, 1	4105838	11p15.1	0.0241
Splicing factor, arginine/serine-rich 8	3352093	12q24.33	0.0243
EST	3503182		0.0244
KIAA0632 protein	3356434	7q22.1	0.0246
Putative translation initiation factor	2823520	17q21.2	0.0248
Ribonuclease H2, large subunit	3160621	19p13.12	0.0248
Adaptor-related protein complex 3, sigma 2 subunit	4139509	15q25.3	0.0254
Regulator of G-protein signalling 14	4547415	5q35.3	0.0254
KIAA1023 protein	37440	7p22.3	0.0257
Chromosome 20 open reading frame 126	4126826	20q11.21	0.0261
MLN51 protein	40515	17q11-q21.3	0.0271
Likely ortholog of mouse synembryon	3633778	11p15.5	0.0274

(continued on next page)

Table 2. (Continued)

Gene Name	Clone ID	Locus	$\alpha$
Ubiquitin-specific protease 11	2961383	Xp11.23	0.0276
v-rel reticuloendotheliosis viral oncogene homolog A*	4547184	11q13	0.029
ATP synthase, H <sup>+</sup> transporting, mitochondrial F0 complex	3826469	7q22.1	0.0291
Hypothetical protein MGC11242	3939938	17q21.32	0.0293
SIN3 homolog A, transcriptional regulator (yeast)	3604034	15q23	0.0293
Dual specificity phosphatase 3	3605391	17q21	0.0298
Asparagine synthetase	3010719	7q21.3	0.03
Copine I	4074508	20q11.21	0.0305
mitochondrial Ribosomal protein L45	3951804	17q21.2	0.0306
RPA-binding transactivator	3926937	19q13.13	0.0308
Hexosaminidase A ( $\alpha$ polypeptide)	3353424	15q23-q24	0.0312
SPRY domain-containing SOCS box protein SSB-3	3342825	16p13.3	0.0319
Steroidogenic acute regulatory protein related	2823277	17q11-q12	0.0319
Chromosome 20 open reading frame 31	3051376	20q11.21	0.036
Enoyl coenzyme A hydratase 1, peroxisomal	4300082	19q13.1	0.036
WD repeat domain 5B	4097434	3q21.1	0.0367
Actin-related protein 2/3 complex, subunit 1B (41 kDa)	3138319	7q22.1	0.037
Epoxide hydrolase 1, microsomal (xenobiotic)	3547085	1q42.1	0.0371
Alkaline phosphatase, placental-like 2	4652865	2q37	0.0376
FtsJ homolog 2 ( <i>Escherichia coli</i> )	3343278	7p22	0.0379
GTP-binding protein 5 (putative)	4564032	20q13.33	0.0386
Inactive progesterone receptor (23 kDa)	3353485	12q13.13	0.0387
EST	4634558		0.0401
FK506-binding protein 8 (38 kDa)	3543042	19p12	0.0421
Thyroid hormone receptor interactor 6	3610413	7q22	0.0421
Tripartite motif-containing 8	4561807	10q24.3	0.0444
<i>RAB4A</i> , member <i>RAS</i> oncogene family	3346455	1q42-q43	0.0455
Effector cell protease receptor 1	3351130	17q25	0.0459
MCM7 minichromosome maintenance deficient 7 ( <i>S. cerevisiae</i> )*	4134871	7q21.3-q22.1	0.0462
ADP ribosylation factor-related protein 1	2989010	20q13.3	0.047
Chromosome 20 open reading frame 35	2959575	20q13.11	0.0473
KIAA0943 protein	3543516	2q37.3	0.0479
Serine/threonine Kinase 15*	3532438	20q13.2-q13.3	0.048
Protein kinase, cAMP-dependent, regulatory, type I, $\beta$	3677877	7pter-p22	0.0485
Hypothetical protein FLJ10468	2822261	1p34.2	0.0495

\*Genes previously shown to be overexpressed in pancreatic cancer by global expression profiling [20–25].

number also showed elevated expression levels in the pancreatic cancer cell lines. For example, several genes at the 19q13.1 amplicon, such as *FBL*, *PD2*, *SUPT5H*, and *SARS2*, were highly expressed in cell lines with increased copy number. None of these genes has been previously linked to cancer, but based on their function, *FBL*, which is known to act in ribosomal RNA processing [38], and *SUPT5H*, a putative modulator of chromatin structure [39], might be implicated in cancer formation.

To evaluate the contribution of gene copy number to gene expression level, a statistical procedure was applied to systematically identify genes whose expression levels were attributable to copy number increase across all 13 cell lines. This analysis revealed 105 genes and included previously described amplified genes as well as known oncogenes, such as *STK15*, *MLN51*, *RAB4A*, and *RELA*. For example, the *STK15* gene (also called *AURKA*, aurora kinase A, and *BTAK*) located at 20q13 is a putative oncogene that has been shown to be amplified and overexpressed in many human cancers [40–42]. *STK15* is essential for normal centrosome function and chromosome segregation [43] and, recently, *STK15* overexpression was shown to be involved in degradation of the p53 tumor-suppressor protein, leading to loss of p53 function [44].

Although gene copy number has been shown to be a major determinant of gene expression level, a large fraction

of genes shows elevated expression levels without any increase in gene copy number [33,45]. It was therefore expected that our statistical approach identified an overlapping but a clearly separate set of genes than those reported in studies where only expression levels were evaluated. A direct comparison between our data and previously published global gene expression analyses [20–25] (<http://www.ncbi.nlm.nih.gov/SAGE/>) revealed that 16% of the 70 known genes identified here have been previously reported to be highly expressed in pancreatic cancer.

Most of the genes identified by the statistical approach in this study had not been previously linked to pancreatic cancer. However, some of them have been implicated in tumor development. For example, *p21-activated kinase 4* (*PAK4*) has been shown to regulate cell migration, cell adhesion, and anchorage-independent growth both in human cancer cell lines and in fibroblasts, suggesting a central role in oncogenic transformation and tumorigenesis [46–48]. To further clarify the possible roles of the 105 genes in pancreatic cancer, we explored their functional characteristics by using information from the SOURCE database (<http://source.stanford.edu>). This analysis showed that 78% of these genes were involved in functions, such as signal transduction, transcription, and DNA replication, which are essential for normal cellular processes but could also be envisioned to have an impact on cancer development. Taken

together, the set of 105 genes identified in this study is likely to represent genes that are activated by increased copy number and might be actively involved in the pathogenesis of pancreatic cancer. However, it has to be noted that it will be essential to validate the results obtained in this study by using uncultured primary pancreatic tumors. Such validation will also be essential to assess the possible clinical significance of the genes implicated here.

In summary, our microarray-based genome-wide copy number survey resulted in the identification of 24 independent regions of copy number increase in 13 pancreatic cancer cell lines. This approach allowed highly accurate mapping of copy number increases with an average resolution of about 300 kb throughout the genome. Parallel expression analysis using an identical cDNA microarray permitted direct comparison between gene copy numbers and expression levels. A statistical evaluation disclosed a set of 105 genes whose expression levels were directly linked to copy number increase, indicating that they are activated through amplification in pancreatic cancer. These results implicate a set of genes that are likely to have an important role in pancreatic cancer pathogenesis.

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