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Genomic DNA-Chip Hybridization Reveals a Higher Incidence of Genomic Amplifications in Pancreatic Cancer than Conventional Comparative Genomic Hybridization and Leads to the Identification of Novel Candidate Genes

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ABSTRACT

Genomic analyses aimed at the detection of high-level DNA amplifications were performed on 13 widely used pancreatic cancer cell lines and 6 pancreatic tumor specimens. For these analyses, array-based comparative genomic hybridization (Matrix-CGH) onto dedicated microarrays was used. In comparison with chromosomal CGH (eight amplifications), a >3-fold number of DNA amplifications was detected ($n = 29$). The most frequent amplifications mapped to 7p12.3 (three pancreatic cancer cell lines and three pancreatic tumor specimens), 8q24 (four pancreatic cancer cell lines and one pancreatic tumor specimen), 11q13 (three pancreatic cancer cell lines and three pancreatic tumor specimens), and 20q13 (four pancreatic cancer cell lines and three pancreatic tumor specimens). Genes contained in the consensus regions were *MYC* (8q24), *EGFR* (7p12.3), and *FGF3* (11q13). In six of seven pancreatic cancer cell lines and pancreatic tumor specimens with 20q13 amplifications, the novel candidate gene *NFAT C2*, which plays a role in the activation of cytokines, was amplified. Other amplifications also affected genes for which a pathogenetic role in pancreatic carcinoma has not been described, such as *BCL10* and *BCL6*, two members of the BCL family. A subset of amplified genes was checked for overexpression by means of real-time PCR, revealing the highest expression levels for *BCL6* and *BCL10*. Thus, Matrix-CGH allows the detection of a high number of amplifications, resulting in the identification of novel candidate genes in pancreatic cancer.

INTRODUCTION

DNA amplification is one of the mechanisms resulting in an elevated gene expression and has been found in a broad spectrum of tumor types (1). In some cancers, amplifications of pathogenetically and clinically relevant genes have been described, *e.g.*, *MYCN* amplifications in neuroblastoma or *HER2/NEU* amplifications in breast cancer (1, 2). By comparative genomic hybridization (CGH) studies, such amplifications of genomic segments mapping to chromosome arms 12p, 16p, 17q, 19q, 20q,and 22q have also been identified in pancreatic carcinoma (3–7). However, due to the limited spatial resolution of this method (>2 Mbp; Ref. 8), these findings have not resulted in the identification of biologically relevant genes. Array-based CGH (Matrix-CGH), a novel genomic screening strategy, has been shown to provide genomic analysis at high accuracy and a 20- to 100-fold higher spatial resolution than conventional CGH (9–12).

Therefore, we have analyzed 13 widely used pancreatic carcinoma cell lines as well as 6 primary tumor (PT) samples using Matrix-CGH. For this study, a dedicated DNA microarray developed for the detection and analysis of novel pathogenetically relevant genes was applied. To evaluate the analytical power of this technique, all samples were also analyzed by conventional CGH. For the assessment of the possible pathogenetic significance of the genomic data, mRNA expression levels for a subset of amplified genes were analyzed by real-time PCR. Using this approach, a number of previously unknown genomic regions possibly involved in the pathogenesis of pancreatic cancer were identified.

MATERIALS AND METHODS

Cell Lines

Thirteen well-established pancreatic carcinoma cell lines (ASPC1, CAPAN-1, HPAF, IMIM-PC1, IMIM-PC2, MIA PACA, PANC1, PA-TU 8902, PA-TU 8988S, PA-TU 8988T, SKPC, SUIT 007, and SUIT 028) were analyzed. The cell lines were obtained from the following suppliers: ASPC1, American Type Culture Collection (Manassas, VA); CAPAN-1, German Cancer Research Center (Heidelberg, Germany); HPAF, R. S. Metzger (Durham, NC); IMIM-PC1, IMIM-PC2, and SKPC, Real F. X. (Institute Municipal de Investigacion Medica, Barcelona, Spain; Ref. 13), PANC1 and MIA PACA, European Collection of Animal Cell Cultures (Salisbury, United Kingdom); PA-TU 8902, PA-TU 8988S, and PA-TU 8988T, H. P. Elsaesser (Department of Anatomy and Cell Biology, University of Marburg, Marburg, Germany; Ref. 14); and SUIT 007 and SUIT 028, Dr. Takeshi Iwamura (Miyazaki Medical College, Miyazaki, Japan; Ref. 15).

PT Samples

Six fresh frozen tissue samples were obtained from patients with histologically confirmed pancreatic cancer who were treated at the Department of General, Visceral, and Vascular Surgery, University of the Saarland (Homburg/Saar, Germany) after informed consent was obtained. Four tissue samples originated from PTs, and two samples (LP1 and LP115) were obtained from liver metastases (LMs). Details are given in Table 1.

Matrix-CGH

Clone Preparation and Spotting. The target clones for the DNA-Chips used in this study were selected based on their chromosomal mapping positions (*a*) covering regions, which are recurrently affected in tumorigenesis or (*b*) containing genes involved in the pathogenesis of many tumors, such as cell cycle regulators, oncogenes, or tumor suppressor genes. The clones were obtained from the libraries RPCIB753 and RPCIP704 at the Human Genome Centre (Berlin, Germany). For analysis of the cell lines, a dedicated microarray containing 498 clones was used (for details, see Ref. 9), which was replaced by a larger chip containing 812 clones for the analysis of the PT samples. Of those 812 clones, 209 clones were uniformly distributed across all autosomes at a distance of about 15 Mbp. Ninety clones covered the sex chromosomes and served as an internal control for the hybridization.

Isolation and spotting of DNA probes were performed as described previously (16), with slight modifications. Briefly, DNA was isolated according to

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Table 1 *Clinical characteristics of the primary tumor samples*

Tumor sample	Age (yrs)	Sex	PT[a]/ LM	TNM stage	Histology
Pp52	87	m	PT	pT_3, pN_0, G I–II	Ductal adenocarcinoma
Pp81	61	m	PT	pT_3, pN_0, G III	Ductal adenocarcinoma
Dp85	70	f	PT	pT_1, pN_1, G II–III	Ductal adenocarcinoma
Dp175	70	f	PT	pT_3, pN_0, pL_1, G II	Ductal adenocarcinoma
Lp1	67	m	LM	pT_4, pN_1, pM_1, G IV	Neuroendocrine adenocarcinoma
Lp115	62	f	LM	pT_3, pN_1, pM_1, GII	Ductal adenocarcinoma

[a] PT, primary tumor; LM, liver metastasis.

standard protocols (Qiagen, Hilden, Germany), sonicated to fragments of 500-5000 bp in size, and dissolved in 3× SSC at a concentration of 0.75 μg/μl in a 384-well plate. Using an Omnigrid microarrayer (Gene Machines, San Carlos, CA), each clone was spotted in eight replicas onto Corning CMT-Gaps II glass slides. After printing, the slides were baked at 80°C for 10 min, exposed to UV light for cross-linking of the DNA (254 nm/2400 μJ), and stored at room temperature.

Labeling and Hybridization. Genomic DNA was isolated using the DNeasy Tissue Kit (Qiagen). Tumor DNA and reference DNA (Human genomic DNA; Promega, Madison, WI) were differentially labeled with Cy3- and Cy5-conjugated dCTP by random primed labeling. After labeling, unincorporated nucleotides were removed using Microcon YM-30 columns. Differentially labeled reference DNA was combined with tumor DNA as well as 70 μg of human Cot-1 DNA. After purification, this mixture was resolved in 120 μl of Ultrahyb buffer (Ambion, Austin, TX) preheated to 68°C. Denaturation was done at 75°C for 10 min, and preannealing was done at 37°C for 30 min. After hybridization for 36 h at 37°C using a GeneTac hybridization chamber (Genomic Solutions, Cambridgeshire, United Kingdom), slides were washed three times in 2× SSC, 50% formamide, and 0.1% Tween 20 (pH 7.0) at 45°C using a flow time of 30 s and a hold time of 3 min. Afterward, slides were washed for 2 min in 1× PBS, 0.05% Tween 20 (pH 7.0) at 25°C. Slides were dried in a centrifuge by spinning for 5 min at 2000 × *g*. Every tumor probe was hybridized twice including a "color switch" of the respective tumor and reference DNA (16).

Image Analysis

Image analysis was performed using a dual laser scanner (Axon 4000B) and GenePix Pro 4.0 imaging software (GenePix 4000 A; Axon Instruments, Union City, CA). The resulting data were analyzed using a dedicated software as described previously (16). Fluorescence ratios were normalized by using the median of the fluorescence ratios computed as $\log_2$ values from the 209 DNA control fragments linearly distributed across the genome. Thereafter, the ratios of the two color-switch hybridizations were averaged and normalized. Based on the ratios of clones mapping to chromosome X in a hybridization of normal male *versus* normal female DNA, a cutoff value of 1.6 for specific amplicon detection was determined (for details, see Ref. 16). The X and Y chromosomes were excluded from the analysis in the PTs because in those experiments male tumor DNA was hybridized with control DNA of a female and *vice versa* to serve as an internal control for the detection of genomic imbalances. This type of internal control hybridizations is not possible in cell lines due to the frequent loss of the Y chromosome (17).

CGH

CGH was performed as reported elsewhere (18). Briefly, tumor DNA was labeled with biotin-16-dUTP (Boehringer Mannheim), and normal human control DNA was labeled with digoxigenin-11-dUTP (Boehringer Mannheim) by a standard nick translation reaction. After hybridization for 1–2 days and posthybridization washes, control and test DNAs were detected via rhodamine and FITC, respectively. Image analysis was performed using an epifluorescence microscope (Axioplan; Zeiss, Jena, Germany) and the commercially available image analysis system ISIS (MetaSystems, Altlussheim, Germany). Overrepresentations were considered as high-level amplifications when the fluorescence ratio values exceeded 2.0 or when the FITC fluorescence showed strong focal signals (19).

Interphase Fluorescence *in Situ* Hybridization (FISH) using Specific DNA Probes

In four PTs (Pp52, Pp81, Dp85, and LP115), FISH analysis was performed to evaluate the specificity of the Matrix-CGH analysis. For this purpose, four DNA probes were selected from the array, which contained the following genes: *Cyclin E1* (used for FISH analysis of case Pp81); *CDK4* (cases Pp81 and LP115); *ERBB2* (cases Dp85 and LP115); and c-*MYC* (case Dp85). Hybridization and evaluation of the experiments were performed as described previously (20).

Real-Time PCR

Real-time PCR was done using the comparative C_T Method according to the instructions of the manufacturer on the ABI Prism 7700 Sequence Detection Systen (PE Applied Biosystems, Foster City, CA). In brief, total RNA was prepared using the RNeasy Mini-Kit (Qiagen, Hilden, Germany), and DNA was removed by DNase digestion using the DNA-free Kit from Ambion. cDNA was reverse transcribed from 5 μg of total RNA using Superscript III Reverse Transcriptase (Invitrogen, Carlsbad, CA) according to the instructions of the manufacturer. Primers for eight genes (*BCL6*, *BCL10*, *EGFR*, *MYC*, *ZNF217*, *BCAS*, and *RPLP0*; see Table 2) were picked using Primer Express 1.5 software

Table 3 *Overview of all amplified regions detected by matrix-CGH[a] in 13 cell lines*

Cell line	Region(s)	Detected by chromosomal CGH
PANC1	1p22	
SUIT 007	2q22–23	
CAPAN-1	2q35–36	
PA-TU8988S	3q27	
CAPAN-1	5p15.2	Yes
SKPCI	7p12.3	
PANC1	7p12.3	
CAPAN-1	7p12.3	
CAPAN-1	7q21.3	Yes
IMIM-PC1	8q24.12–13	Yes
PA-TU 8902	8q24.12–13	
HPAF	8q24.12–13	
PA-TU8988T	8q24.12–13	
IMIM-PC1	10q22.2/10q25.3–26.1	Yes
HPAF	11q12–13	Yes
SUIT 028	11q12–13/11q24	
SUIT 007	11q12–13/11q23–24	
PA-TU 8902	12p13	Yes
SUIT 028	12q13–15	
IMIM-PC1	13q14	
IMIM-PC1	14q32.32	
SKPC I	18p11.22	
PA-TU8988T	18q11.2	
PA-TU8988S	18q11.2	
8988T	20q13.1	Yes
8988S	20q13.1	
SUIT 028	20q13.1	
SUIT 007	20q13.1	
SUIT 028	Xq22.2b–3a	Yes

[a] CGH, comparative genomic hybridization.

Table 2 *Primers used for real-time PCR analyses*

Gene	Primer forward	Primer reverse
BCAS	5′-CCC-CGA-GAC-AAC-GGA-GAT-AA	5′-CTC-GGG-TTT-GGC-CTC-TTT-C
BCL6	5′-CTC-CGT-GCC-CAT-GTG-CTT-A	5′-GAG-TCT-GAA-GGT-GCC-GGA-AA
BCL10	5′-GTG-TGC-CAC-CAT-GCC-TCA-C	5′-ACG-CTG-GGT-AGG-TTG-CTT-GA
EGFR	5′-GCG-TCT-CTT-GCC-GGA-ATG-T	5′-GGC-TCA-CCC-TCC-AGA-AGC-TT
MYC	5′-TAG-GCG-CGC-GTA-GTT-AAT-TC	5′-CGG-AGA-GTT-AGC-GAG-AGA-GG
RPL P0	5′-AGA-TCC-GCA-TGT-CCC-TTC-G	5′-CCT-TGC-GCA-TCA-TGG-TGT-T
ZNF217	5′-GTG-GCC-TTA-TTC-CAA-CTC-CA	5′-GGG-CAT-ATA-CCC-CTC-GAT-TT

Table 4 *Regions not previously described to be amplified in pancreatic cancer*

Chromosomal region	Possible target genes
1p22	*BCL10*
2q22–23	*SIP1*
2q35–36	*WNT10A/WNT 6*
3q27	*BCL6*
12q13–15	*MLL2/SAS/CDK4/MDM2*
13q14	*RB1*
14q32.32	*AKT1*
18p11.22	*PPP4R1*
18q11.2	*LAMA3*
Xq22.2	*RAB40A*

(PE Applied Biosystems). Samples (2.5% of the reverse transcription reaction) were amplified using the SYBR Green PCR Master Mix system (PE Applied Biosystems) and cycling conditions of 15 s of denaturating time (95°C) and 1 min of annealing/amplification time (60°C) for 40 cycles after an initial activation step of 10 min at 95°C. The human *RPLP0* gene (ribosomal protein large P0; GenBank accession no. BC 001127) is used as an internal standard because it has been shown not to be differentially regulated between normal and cancerous tissue of the pancreas (21) and was measured in duplicates, whereas all other genes were measured in triplicates. The mean SD for all replicates was 0.2.

RESULTS

Matrix-CGH

Cell Lines. In cell lines 29 high-level amplifications were detected by Matrix-CGH in contrast to only 8 by conventional CGH. The most frequently amplified regions mapped to chromosome arms 20q (31%, 4 of 13), 8q (31%, 4 of 13), 11q (23%, 3 of 13), and 7p (23%, 3 of 13). These regions covered genes already discussed to be involved in the pathogenesis of pancreatic carcinoma such as c-*MYC* (8q24), *cyclin D1* (11q13), or *EGFR* (7p12). A total of 20 amplified chromosomal regions (see Table 3) were identified. In addition to 10 regions (5p15, 7p12, 7q21, 8q24, 10q22, 10q25, 11q12q13, 11q24, 12p13, and 20q13) already known to be amplified in pancreatic cancer or pancreatic cancer cell lines (3, 5, 6, 7), 10 regions not yet known for their involvement in the pathogenesis of pancreatic carcinoma could be described. Table 4 gives an overview of the newly discovered regions and possible target genes.

PTs/Liver Metastases. Regions commonly amplified in cell lines such as 7p12, 8q24, 11q12q13, and 20q31 were also found to be amplified in PTs. Regions affected in at least three carcinomas were

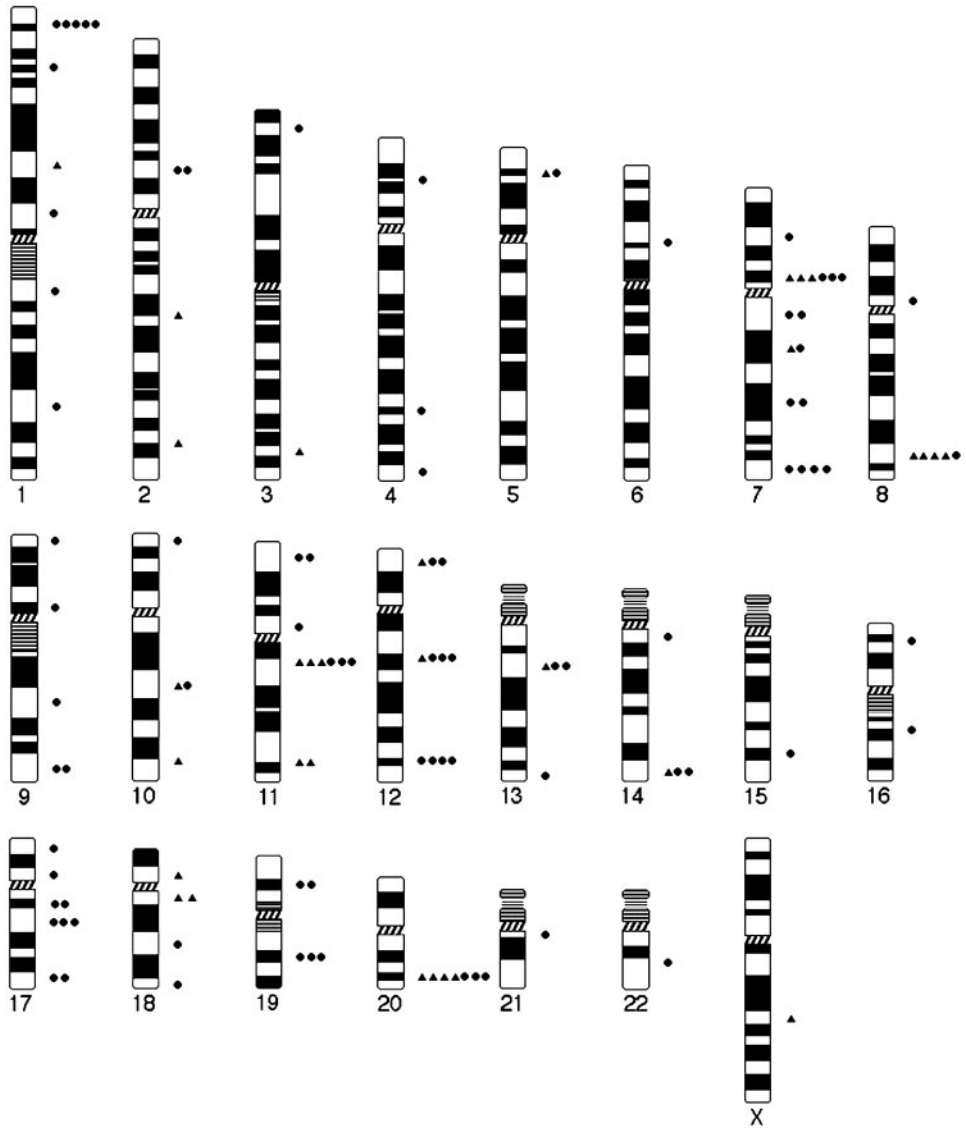

Fig. 1. Schematic illustration of DNA amplifications found in cell lines (▲) and primary tumors (●).

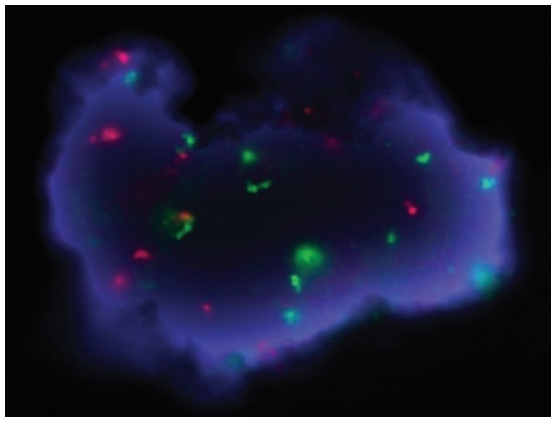

Fig. 2. Fluorescence *in situ* hybridization of probe N2031 (containing the *CCNE1* gene, *green*) and probe J15872 (containing the *CDK4* gene, *red*) to cell of tumor Pp52. For both probes, >5 fluorescence signals can be detected, confirming the amplifications of both genes identified by array CGH.

7q36 (three of four PTs and one of two LMs), 7p12 (one of four PTs and two of two LMs), 12q13 (one of four PTs and two of two LMs), 17q21 (one of four PTs and two of two LMs), and 20q13 (two of four PTs and one of two LMs). The DNA amplifications most frequently amplified in cell lines and PTs are illustrated in Fig. 1.

Interphase FISH

To evaluate the specificity of the Matrix-CGH analysis in pancreatic carcinoma, eight gene amplifications detected by Matrix-CGH in four PT tissues were also analyzed by interphase FISH: *ERB-B2* in cases Dp85 and LP115; *MYC* in Dp85; *CCDN1* in Dp81 and Pp81; and *CDK4* in Dp81 and LP115. In all eight cases, a signal pattern characteristic of gene amplification was identified in >20% of cells (for an example, see Fig. 2).

Delineation of Consensus Regions

Four amplified genomic regions were detected in at least 5 of the 19 tumor cell lines and tissue samples: 7p12; 8q24; 11q12q13; and 20q13. For bands 7p12 and 8q24, the spatial resolution of our array was >10 Mbp. However, in all six cases with 7p12 amplifications, clones containing the *EGFR* gene were affected. Similarly, in all five cases with 8q24 amplifications, target clones containing the c-*MYC* gene were amplified. For bands 11q12q13 and 20q13, there was a higher density of target clones. The results for these regions were as follows.

11q12-q13. On band 11q12-q13, two consensus regions were delineated. *MAP3K11* was affected in all five cases with 11q amplifications. For another clone containing the *cyclin D1* gene, gains were identified in six cases, three of which fulfilled the criteria for DNA amplification (see Table 5A).

20q13. The most frequently amplified regions mapped to chromosome band 20q13. On the array, eight target clones mapped to chromosome arm 20q. The most frequently amplified clones were RP11-24K24 and RP11-924D88, which are located between the *PTPRT* and *ZNF217* genes. If all genomic gains are considered, the consensus region extends from clone RP11-151D28 to clone RP11-973G14, which contains the *ZNF217* gene (see Table 5B). Possible candidate genes in those regions are *PTPRT* (protein tyrosine phosphatase, receptor type, T), *BCAS* (breast carcinoma amplified sequence 1),

Table 5 *Delineation of consensus regions*

A) Patter of genomic aberratins on chromosome arm 11q

	Localization 11q	Pp 52	Lp1	Lp115	Suit 007	Suit 028	HPAF
	Mbp from 11 cen						
	61,6						
	62,85						
	64,45						
	65,52						
MAP3K11 →	65,61						
	65,85	/		/		/	
	66,96						
	69,68						
CyclinD1 →	69,71						
	69,8						
	69,85			/			
	72,16						
	76,12				/	/	
	84,91						
	93,1	/	/	/			
	110,45						

B) Pattern of genomic aberratins on chromosome arm 20q

	Localization 20q	8988 T	8988 S	Suit 007	Suit 028	Dp 85	Dp 175	Lp 1
	Mbp from 20cen							
	30,7							
PTPRT →	41,9	/	/	/	/			
	47,5	/	/	/	/			
AIB1 →	45,9							
NFAT C2 →	49,7							
ZNF217 →	51,8			/	/			
BCAS →	52,3							
	54,6	/	/	/	/			

*In many CGH studies, ratio values exceeding 1.25 have been used for the identification of genomic gains (*e.g.*, Ref. 4). Therefore, clones with ratio values >1.25 are indicated as ″gains″ in these tables.

ZNF217 (zinc finger protein 217), *AIB1* (amplified in breast cancer 1), *NFAT C2* (nuclear factor of activated T cells), *OVCOV1* (ovarian cancer overexpressed 1), and *TDE1* (tumor differentially expressed 1).

Real-Time PCR

To compare the expression level of some of the amplified genes in PTs and cell lines, a subset of five genes was measured in comparison with the ribosomal gene *RPLP0* in eight cell lines (PA-TU 8988S, PA-TU 8988T, PANC1, IMIM-PC1, IMIM-PC2, HPAF, SUIT 007, and SUIT 028) and four PTs (Pp81, Dp85, LP1, and LP115). All genes revealed comparable expression levels in tumors as well as in the cell lines, indicating that the selected amplified genes are not only comparable with respect to their amplification status but are also comparable with respect to their expression level (Fig. 3*A*).

To evaluate a possible differential expression between tumor and healthy tissue, a real-time PCR was performed on three PTs and three microscopically normal pancreatic tissues obtained from the resection margins of pancreatic cancer samples. *BCL6*, *BCL10*, and *ZNF217* were analyzed using real-time PCR: for *BCL10* and *ZNF217*, an overexpression was identified in the tumor samples (Fig. 3*B*), which has not been described before. For *BCL6*, there was also a higher expression in the tumor samples, which, however, was less pronounced. Taking into account the possibility that the resection margins might be contaminated with some tumor cells, it is still possible that a differential expression of *BCL6* is present in the tumor tissues.

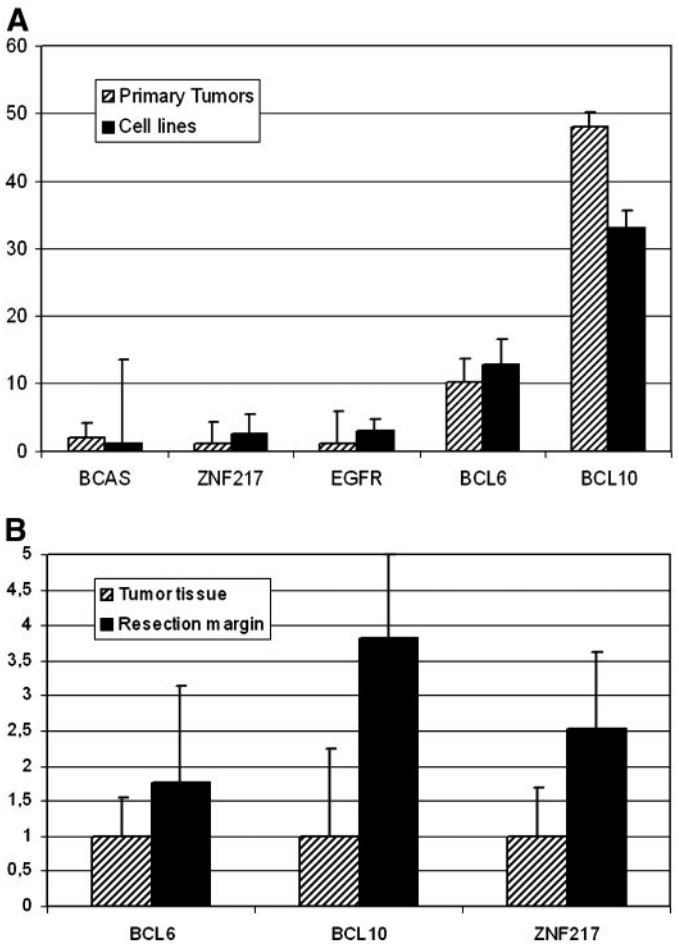

Fig. 3. *A*, mean relative expression levels for selected genes in eight pancreatic cancer cell lines and four primary tumor samples. Note the similar expression levels in cell lines and tumor samples. The *error bars* indicate 1 SD. *B*, mean relative expression levels for *BCL6*, *BCL10*, and *ZNF217* in three primary tumor tissues compared with three nontumorous pancreatic tissues obtained from resection margins. The *error bars* indicate 1 SD.

DISCUSSION

Oncogene amplification is a major genomic force contributing to the development of human tumors (2), and the importance of such amplifications has been demonstrated in several hundred studies (22). In contrast to many other tumors, little is known about oncogene amplifications and other genomic aberrations in pancreatic cancer. By chromosomal banding analysis, highly complex karyotypes were identified in pancreatic cancer samples. Nevertheless, only a few recurrent aberrations have emerged from these studies (23–25). By CGH studies, several typical regions with genomic gains have been identified. These include 8q, 11q, 16p, 17q, 20q, and 22q (3, 4, 6, 7). Because those CGH studies often revealed large and insufficiently characterized regions, we initiated a high-resolution array CGH analysis of 13 widely used pancreatic cancer cell lines. In contrast to our CGH data, we identified approximately three times as many amplifications using array CGH. The high specificity of array CGH was demonstrated by FISH experiments to interphase nuclei. In addition to chromosomal segments already known to be amplified in pancreatic carcinoma, we discovered several amplifications that were previously not recognized as recurrent aberrations in this tumor. The most frequent amplifications were also identified in six PT samples, confirming that the relevance of these findings is not limited to cell lines.

To test the biological relevance of the genomic aberrations, expression studies using real-time PCR were performed for a subset of amplified genes. The highest expression levels were found for two members of the BCL family, *BCL10* and *BCL6*, which have not been described previously in the context of pancreatic carcinoma. High expression levels in all analyzed cell lines and tumor samples suggest a role for these genes in the pathogenesis of pancreatic cancer. *BCL10* was differentially expressed in the tumor tissue compared with the resection margins. This gene is overexpressed in some MALT lymphomas as a consequence of the chromosomal translocation t(1,4)(p22,q32) (26). Such an overexpression has been discussed to result in nuclear factor-κB-mediated inhibition of apoptosis (27). The *BCL6* proto-oncogene encodes a transcriptional repressor and is frequently deregulated by genomic aberrations in B-cell lymphomas (28). Overexpression of this gene not only immortalizes primary mouse embryonic fibroblasts and cooperates with *RAS* in oncogenic transformation but also overrides the senescence response downstream of *p53* (29). Additionally, *BCL6* overexpression in cell lines is able to significantly inhibit apoptosis caused by etoposide and other chemotherapeutic agents (30). Recently, amplification and overexpression of *BCL6* have been described in another epithelial cancer (31), indicating that the pathogenetic relevance of this gene is not restricted to B-cell lymphomas.

Apart from the identification of additional aberrations, the superior spatial resolution of array CGH also allows a detailed fine mapping of amplified regions (see Table 5, A and B). For band 11q12-q13, a commonly amplified region was identified containing the *MAP3K11* gene. This gene encodes a widely expressed protein kinase (32). Another gene with frequent copy number increases is *cyclin D1*, which is already suspected to be involved in pancreatic cancerogenesis (33).

Chromosomal band 20q13 was the most frequently amplified region with aberrations extending over 15–20 Mbp. This region has been analyzed in other studies, and *AIB1* (34) and *CTSZ* (cathepsin Z; Ref. 7) were identified as frequently amplified genes. In our series, a number of genes were part of the consensus region (*BCAS*, *ZNF217*, *AIB1*, *OVCOV1*, *TDE1*, and *NFAT C2*). *CTSZ* was not part of the consensus region. *BCAS1* is a novel gene that was found to be

amplified and overexpressed in breast cancer (35). To our knowledge, no expression of *BCAS* is found in normal pancreatic tissue. *ZNF217* is a zinc finger protein and has also been found to be amplified and overexpressed in breast cancer (35, 36), ovarian cancers (37), and prostate cancer (38). It has also been shown that ZNF217-transduced cell cultures gave rise to immortalized cells (39). Both genes were found to be expressed in all cell lines and PTs, and for ZNF217, we could demonstrate an overexpression in pancreatic tumors. *OVCOV1* is a gene that was found to be overexpressed in ovarian cancer (40), but it has not yet been connected to any other tumor type. Increased expression of the mouse testicular tumor differentially expressed (*TDE*) gene is found in murine testicular tumors as well as testicular tumor cell lines. Elevated expression of the human homolog gene was observed in three of five lung tumors (41). Receptor protein tyrosine phosphatase rho (RPTPrho, gene symbol *PTPRT*) is a member of the type IIB RPTP family. These transmembrane molecules have been linked to signal transduction, cell adhesion, and neurite extension (42) The most frequently amplified gene in this region was *NFAT C2*, which was amplified in six of seven pancreatic tumor samples with 20q13 amplification. This gene plays a role in the activation of cytokines (43). In addition, genes of the *NFAT* family have recently been shown to be involved in the promotion of carcinoma invasion (44).

These data underline the high sensitivity of Matrix-CGH for the identification of gene amplifications in pancreatic cancer. In addition, a fine mapping of aberrations is facilitated, which allows the rapid identification of candidate genes. These findings can be used as starting points for a more focused investigation of pathobiology in this tumor type.

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