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Original article

Losses at chromosome 4*q* are associated with poor survival in operable ductal pancreatic adenocarcinoma

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A B S T R A C T

Here we tested the prognostic impact of genomic alterations in operable localized pancreatic ductal adenocarcinoma (PDAC). Fifty-two formalin-fixed and paraffin-embedded primary PDAC were laser micro-dissected and were investigated by comparative genomic hybridization after whole genome amplification using an adapter-linker PCR. Chromosomal gains and losses were correlated to clinico-pathological parameters and clinical follow-up data. The most frequent aberration was loss on chromosome 17*p* (65%) while the most frequent gains were detected at 2*q* (41%) and 8*q* (41%), respectively. The concomitant occurrence of losses at 9*p* and 17*p* was found to be statistically significant. Higher rates of chromosomal losses were associated with a more advanced primary tumor stage and losses at 9*p* and 18*q* were significantly associated with presence of lymphatic metastasis (chi-square: $p = 0.03$, $p = 0.05$, respectively). Deletions on chromosome 4 were of prognostic significance for overall survival and tumor recurrence (Cox-multivariate analysis: $p = 0.026$ and $p = 0.021$, respectively). In conclusion our data suggest the common alterations at chromosome 8*q*, 9*p*, 17*p* and 18*q* as well as the prognostic relevant deletions on chromosome 4*q* as relevant for PDAC progression. Our comprehensive data from 52 PDAC should provide a basis for future studies with a higher resolution to discover the relevant genes located within the chromosomal aberrations identified.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the predominant histological type among pancreatic malignancies and with a 5-year survival rate of 3–5%, considered as one of the most lethal cancer types [1,2]. At diagnosis, most patients present with locally advanced, metastatic disease rendering their cancers incurable. Even in selected patients who received surgical treatment with curative intention, the reported 5-year survival rates range around only 20% [3,4]. Residual tumor at the resection margin (*R*1) has been discussed as one reason for this extremely poor prognosis [4–6]. However, the survival of patients with tumor free resection margins (*R*0), when checked by a thorough pathological work-up protocol, is not dramatically better [5] questioning this hypothesis. The tumor-node-metastasis (TNM) staging system is currently the method of choice to separate resectable patients with early

Abbreviations: PDAC, pancreatic ductal adenocarcinoma; CIN, chromosomal instability; CGH, comparative genomic hybridization; mCGH, metaphase CGH; SCOMP, single cell comparative genomic hybridization; SD, standard deviation; n.s., not significant; vs, versus; *n*, variable quantity.

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tumor stage and a possibly better prognosis from the group of patients with advanced lesions that are considered incurable and not resectable [7]. Nonetheless, individual outcome prediction is uncertain, since patients with an identical primary tumor stage can experience significantly different disease outcomes. Therefore, a further biological characterization of PDAC might provide additional prognostic information for an improved treatment selection, e.g. for multimodal therapeutic approaches [8,9] or for molecular therapies [10].

Sporadic PDAC is characterized by marked chromosomal instability (CIN) and several data indicate that CIN can be regarded as one of the driving forces for PDAC development and progression [1,11–16]. CIN refers to an accelerated rate of gains or losses of whole or large portions of chromosomes that results in karyotypic variability from cell to cell [17,18]. Consequences of CIN are imbalance in chromosome number (aneuploidy) and structural chromosomal alterations. Telomere attrition is believed to be a major promoter and potential initiator of CIN leading, among other chromosomal changes, to amplifications and deletions at the sites of chromosomal rearrangements as well as at the points of chromosomal breakage through the development of breakage-fusion-bridge (BFB) cycles [19–21]. Interestingly, global telomere erosion is already present in over 90% of early precursor lesions (PanIN-1A) and seems to even precede the development of mutations in the *KRAS* and *TP53* genes [22]. In addition to centromere amplifications, defects of the spindle apparatus seem to contribute to CIN as well and have been frequently observed in PDAC [23,24]. In this context, we were interested whether CIN could be used as prognostic marker. Thus, we investigated the ploidy levels of malignant pancreatic ducts using chromogenic in situ hybridization and observed that high levels of aneuploidy conferred a higher risk for early metastatic relapse as well as for tumor related death [25]. This finding was supported by similar observations of other groups [26,27]. Surprisingly, the clinical relevance of global chromosomal imbalances is thus far poorly investigated in PDAC. In order to assess such global genomic imbalances in primary PDAC, we used Comparative Genomic Hybridization (CGH) to screen for genome-wide chromosomal gains and losses. Since PDAC exhibit a complex morphology, we performed laser-assisted micro-dissection for the isolation of malignant ducts from the surrounding fibrous tissue prior to Single cell COMParative genomic hybridization (SCOMP) for representative whole genome amplification. SCOMP is an adapter-linker PCR approach for single cell amplification that has repeatedly been shown to be superior to other whole genome amplification methodscommonly used for few cell amplification and subsequent CGH analysis from formalin-fixed and paraffin-embedded tissues [28,29]. The CGH results retrieved in our study were then used to search for association with clinicopathological factors and tumor-specific survival data to test their prognostic significance.

2. Materials and methods

2.1. Patients and tumor samples

All samples were derived from formalin-fixed and paraffin-embedded tissue (FFPE) blocks from routine histopathology of 52 patients who underwent partial pancreatoduodenectomy and radical lymphadenectomy with curative intention (*R*0) at the University Hospital Hamburg-Eppendorf (Table 1). Clinicopathological data were acquired with approval of the ethics committee of the Hamburg Chamber of Physicians, Germany.

The median age of the patients was 60.2 years (range 33–82 years). Twenty-three patients were females (44%) and 29 males (56%). TNM classification and staging was performed according to the sixth edition of the UICC (International Union against Cancer) guidelines [30]. One tumor was classified as *p*T1 (2%), 25 as *p*T2 (48%), 25 as *p*T3 (48%), and one as *p*T4 (2%). Thirty-three patients (63%) had primary lymph node metastases (*p*N1) while patients with initial distant metastases (M1) were not integrated into our study. Twenty-seven tumors (52%) were categorized as G2 and 25 as G3 (48%). Clinical follow-up data were available for 50 patients. None of the patients included into the survival analysis received pre-operative (radio-)chemotherapy. Two patients died of non-tumor related death during the hospital stay and were therefore not included in our outcome analysis. The median clinical observation period was 14.5 months.

Table 1
Demographic as well as clinico-pathological characteristics of our patient population.

Parameters	Category	*n* (%)
Gender	Female	23 (44)
	Male	29 (56)
Age	Median [range]	60,2 years [33–82]
Depth of invasion	*p*T1	1 (2)
	*p*T2	25 (48)
	*p*T3	25 (48)
	*p*T4	1 (2)
Lymph node involvement	*p*N0	19 (27)
	*p*N1	33 (63)
Grade of differentiation	G2	27 (52)
	G3	25 (48)
UICC Stage	I	13 (25)
	II	6 (12)
	III	32 (62)
	IV[a]	1 (2)

[a] UICC IVA – No distant metastasis (pT4).

2.2. Microdissection

First, a pathologist (A.E.) reviewed the specimen and selected suitable FFPE tissue blocks. Subsequently, sequential 5-μm sections were cut from the selected FFPE tissue blocks using a microtome. For morphological control, one slide was stained with conventional hematoxylin and eosin staining and the sequential section was prepared for laser micro-dissection and mounted onto a 1.35-μm-thin polyethylene membrane (P.A.L.M. Microlaser Technologies, Bernried, Germany), attached to a glass slide. For micro-dissection the tissue sections were deparaffinized on a shaker, changing the xylene twice, incubated for 30 min each and were finally rehydrated with a series of 100%, 85%, and 70% ethanol. To avoid interference of the nuclear staining with the PCR amplification, slides were stained in diluted (50%) hematoxylin (Gill's, Sigma, St. Louis, MO, USA) for 5 min. The staining was followed by a dehydrating ethanol series and the slides were dried overnight in the presence of a desiccant. For micro-dissection we used the P.A.L.M. Laser-Microbeam system (P.A.L.M. Microlaser Technologies, Bernried, Germany). The inner side of a 200-μl tube cap was covered with 3–5 μl of PCR oil and the isolated cells were catapulted into the cap (Fig. 1A). The cap was subsequently mounted onto the tube and centrifuged at 14,000 μg for 5 min. Then 3 μl of lysis buffer [10 mmol/L Tris-acetate, pH 7.5, 10 mmol/L Mg-acetate, 50 mmol/L K-acetate (0.2 μl of 10X Pharmacia One-Phor-All-Buffer-Plus)], 0.67% Tween 20 (Sigma, Deisenhofen, Germany), 0.67% Igepal (Sigma), and 1.3 mg/ml proteinase K were added to the tube and centrifuged again for 14,000 μg for 5 min to separate the reaction mix from the oil.

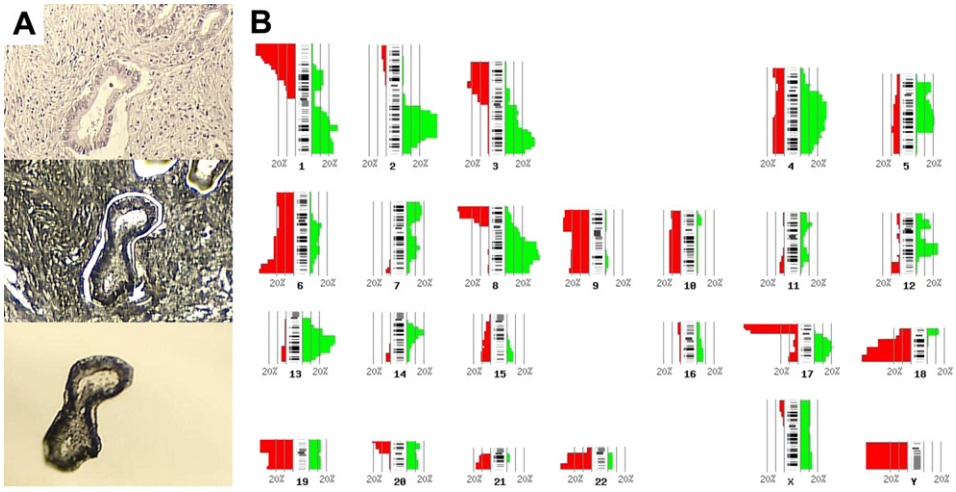

Fig. 1. A: Laser-assisted micro-dissection sequence of a malignant duct in PDAC. B: Histogram that summarizes the chromosomal gains (green) and losses (red) detected in 52 laser micro-dissected PDAC tissues. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.3. SCOMP (Single cell COMParative genomic hybridization)

To apply SCOMP on laser-microdissected cells from FFPE material, it was carried out exactly as described in the protocol by Stoecklein et al. [28]. The labeling reaction was performed in a total volume of 40 μl consisting of 4 μl of 10× PCR buffer (Expand Long Template PCR System, Buffer 1; Roche, Mannheim, Germany), 6 μl of Lib1-primer (10 μmol/L), 1.4 μl of a dNTP stock solution (10 mmol/L for dATP, dCTP, and dGTP; 8.75 mmol/L for dTTP), 1.75 μl of 1 mmol/L digoxigenin-11-dUTP (Roche) for the test-DNA or 1.75 μl of 1 mmol/L biotin-16-dUTP (Roche) for the reference DNA, respectively, and 2.5 U of Taq-polymerase (Roche). We used 1.25 μl of the SCOMP product for reamplification and a MJ-Research PTC-200 thermocycler (Waltham, MA) was programmed to 94 °C (1 min), 60 °C (30 s), 72 °C (2 min) for one cycle; 94 °C (30 s), 60 °C (30 s), 72 °C (2 min plus 20 s/cycle) for 10 cycles. The labeled products were used for CGH as described [28]. Quantitative evaluation of the ratio of test and control DNA was done according to du Manoir and colleagues [31] by using the Leica (Bensheim, Germany) software package Q-CGH. Five to 12 metaphases were evaluated in each experiment.

2.4. Statistical methods

For the statistical computations we used the SPSS 12.0 software (SPSS Inc., Chicago, IL, USA) and the statistical package R [32]. For generation of aberration status matrix and chromosomal ideograms we used Progenetix [33]. To test the associations between non-parametric data (chromosomal aberration number, chromosomal gain number, chromosomal loss number) and clinicopathological variables, we used the Mann–Whitney-*U* test. The two-sided Fisher's exact test, and whenever appropriate the Chi-square test, were used to test for associations between genomic changes as well as between genomic changes and clinicopathological parameters. Tumor-specific survival was estimated using the Kaplan–Meier method. Differences in survival were analyzed regarding clinicopathological features (i.e. age, gender, tumor invasiveness, presence of lymph node metastases as well as tumor grading) as well as regarding chromosomal aberrations by means of log-rank test. The overall survival was computed as the time period from the date of surgery to either the date of death or last follow-up, whichever occurred first. The disease-free survival was defined as the time period from the date of surgery to the date of recurrence, last follow-up or date of death, whichever occurred first. Patients alive without recurrence at the last follow-up dates were censored. Cox proportional hazard model was fitted for multivariate analysis. Differences between groups were considered significant if the p-value was <0.05 in a two-tailed test.

3. Results

3.1. Genome-wide imbalances in PDAC

In our PDAC samples, we detected a mean chromosomal aberration number of 12.2 (SD ± 4.9, range 3–22). In average, 5.8 chromosomal gains were found per patient (SD ± 3.5, range 1–16) with the most frequent gains being located at 2*q* (41%) and 8*q* (41%). Mean chromosomal loss in our cohort was 6.4 (SD ± 2.8, range 1–16). The most frequent loss was observed at chromosome 17*p* (65%) (Table 2, Fig. 1B). No significant difference between the number of chromosomal gains and losses was detected (Mann–Whitney-*U* test, $p = 0.34$). Generally, the histogram of summarized CGH results (Fig. 1B) indicates a high rate of selected alterations, since fourteen aberrations were frequently recurrent and observed in at least 30% of the investigated PDACs (Table 2). We then tested for recurrent combined chromosomal aberrations and observed a significant correlation between the occurrence of 9*p* losses and 17*p* losses (Fisher's exact test: p = 0.03), suggesting

Table 2
CGH aberrations observed in ≥30% of patients.

Chromosome, Arm, Band	Percentage with loss
17*p*11–13	65%
18*q*12–23	58%
1*p*33–36	46%
6*q*24–27	40%
19*p*11–13	39%
8*p*21–23	37%
22*q*	37%
9*p*11–21	31%
	Percentage with gain
2*q*22–34	40%
8*q*21–24	40%
13*q*21–31	38%
3*q*25–27	35%
1*q*31	31%
4*q*21–28	31%

combined losses of *TP53* (on 17*p*) and *INK4A* (on 9*p*). Another frequently amplified region was 8*q*, comprising the MYC oncogene locus. However, studies with a higher resolution are inevitable to test these links in order to prove any such relation. Although, 78% ($n = 18$) of the cases with 8*q* gain displayed losses at 17*p*, no statistical significant correlation was observed between 8*q* gains and losses of 9*p* and 17*p*, respectively (Fisher's exact test: $p =$ n.s.). No further significant recurrent combined chromosomal aberrations were detected.

3.2. Correlation of CGH aberrations with clinico-pathological parameters

We compared the number of chromosomal aberrations with the pT-category, the pN-category and grading, respectively, to check whether local and regional tumor progression correlate with the level of structural genomic instability. We did not observe a statistical significant correlation between an increase of chromosomal aberrations and advanced pT-, pN-category or grading, respectively. However, we found a relationship between a higher number of chromosomal losses and more advanced pT-category (Mann–Whitney-*U* test: $p = 0.03$) as well as a trend for the correlation between a higher number of chromosomal losses and the presence of lymph node metastases (Mann–Whitney-*U* test: $p = 0.09$).

In the next step, we tested whether specific chromosomal aberrations were associated with clinico-pathological factors. We observed that losses at the chromosomal region 9*p*11–21 were significantly correlated with the presence of lymphatic metastasis. Fifteen (83%) of 18 patients with 9*p* losses compared to 16 (47%) of 34 patients without 9*p* loss exhibited lymph node metastasis (Chi-square test, $p = 0.025$). Moreover, we measured a borderline statistical significant correlation of 18*q* losses and lymphatic metastasis. While 23 (74%) of 31 patients with 18*q* losses showed lymph node metastases (*p*N1), only 10 (47%) of the 21 patients without chromosome 18q losses exhibited lymphatic spread (Chi-square test, $p = 0.05$). Otherwise we did not observe any statistical significant correlation between CGH-alterations and standard risk factors.

3.3. Correlation of CGH aberrations with tumor-specific survival

Survival analysis was conducted for 50 cases, since two patients died during the hospital stay, unrelated to PDAC. As expected, the survival analysis revealed lymph node metastases (log-rank: $p = 0.001$) as significant prognostic factor. Primary tumor size or grading was not significantly correlated to survival in our patient cohort. We the then tested whether any of the chromosomal aberrations were of prognostic significance. The 362-band matrix was reduced to a matrix consisting of only *p* and *q* arms of each chromosome (41 variables; alterations on chromosome 19 and Y were excluded from the analysis and for the acrocentric chromosomes 13,14 and 15, respectively, only q-arms were analyzed. The GC-rich chromosome 19 was excluded because alterations – especially deletions – are difficult to interpret in mCGH, since color-switch experiments or further FISH-validation were omitted [34,35]). We analyzed the prognostic relevance of gains and losses using log-rank test. None of the frequent chromosomal aberrations that were detected in at least 30% of the PDAC cases displayed any significant correlation to survival. Among all alterations, only deletions at chromosome 4 were of prognostic significance in the univariate analysis for overall survival ((log-rank: $p = 0.03$ for 4*p* loss); log-rank: $p = 0.009$ for 4*q* loss) (Table 3, Fig. 2). When we corrected the log-rank-data from all 41 variables for multiple testing using the Benjamini and Hochberg false discovery rate, the deletions at chromosome 4 lost their prognostic significance ($p = 0.37$). Despite this observation we entered deletions at chromosome 4 – as identified by the unadjusted univariate analysis – into a multivariate Cox-regression model to test for independent prognostic significance of this factor in our collective. In fact, losses on chromosome 4*q* and lymph node metastases were found to be of independent prognostic significance (Table 4), while no significant correlation was observed for losses on chromosome 4*p*. Interestingly, losses on chromosome 4*q* as well as lymph node metastases also predicted tumor recurrence independent of other factors included into the multivariate analysis (Table 4)

4. Discussion

Very aggressive biological behavior resulting in high mortality rates even after radical resection of the primary tumor and marked chromosomal instability are major hallmarks of pancreatic ductal adenocarcinoma (PDAC). In the present study we tested in an exploratory manner whether genome-wide chromosomal alterations detectable by conventional metaphase-based CGH were associated with tumor-specific survival. Only losses at chromosome 4*q* were found to be of independent prognostic significance with the note of caution that the identified prognostic alteration at 4*q* did not pass the test to correct for multiple testing. Clearly, the prognostic significance of chromosome 4 losses warrants further validation in independent PDAC collectives, e.g. by using FISH probes for the deleted chromosomal region. With a frequency of 16%, the losses of chromosome 4*q* were relatively rare in our collective. However, this frequency is compatible with previous CGH studies in PDAC [36]. Because of the low resolution of conventional CGH and the further reduction in resolution for our survival analysis, clearly, our approach is not suitable to identify cancer related genes within the candidate regions. Losses at chromosome 4 involving large parts of the chromosome, have been previously reported to be of prognostic significance in oral cancer [37], colorectal cancer [38] and hepatoblastoma [39]. Furthermore, deletions at 4*q*12–*q*32 in primary lung cancer were significantly associated with the presence of disseminated tumor cells in bone marrow, indicating that this alteration might be relevant for systemic disease progression [40]. The majority of available CGH

Table 3
Statistically relevant aberrations within univariate survival analysis (log-rank test).

Aberration	Number of patients	Number of tumor-related deaths	Censored patients	Median survival (months)	*p*-value (log-rank test)
4*q* loss					
No	42	22	20	17	0.009
Yes	8	7	1	10	
4*p* loss					
No	43	23	20	16	0.043
Yes	7	6	1	10	

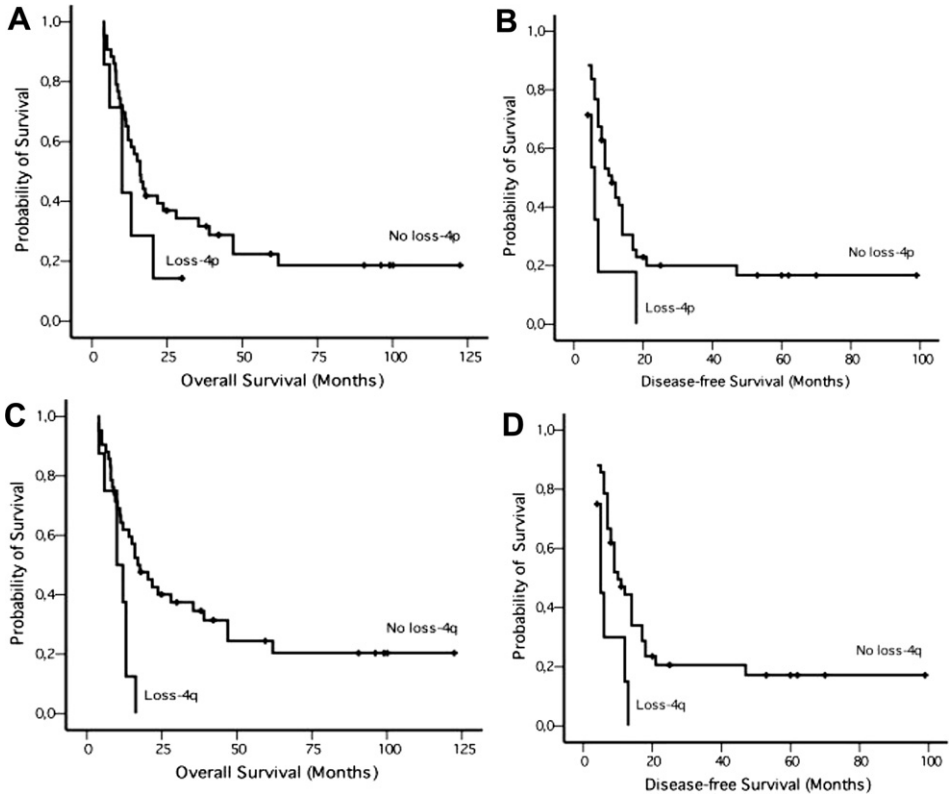

Fig. 2. Kaplan–Meier-curves for overall survival (OS) and disease-free (DFS) survival after curative resection in 50 PDAC patients. Impact of chromosome 4*p* losses on OS (A) and DFS (B). Impact of chromosome 4*q* losses on OS (C) and DFS (D).

studies in PDAC provide a general description of chromosomal imbalances mainly in locally advanced and metastatic cancers (UICC Stages IVa and IVb; Table 5). Furthermore, the published studies often rely on a small heterogeneous sample size. Only very few authors such as Schleicher et al. correlated the CGH results in PDAC with clinical outcome [41]. They identified chromosome 8*q* gain as a prognostic factor for poor survival. However, of the 32 investigated pancreatic cancers, only 25 tumors displayed PDAC morphology. Furthermore, micro-dissection was not performed, which reduces the sensitivity of CGH and may explain the relatively low reported mean aberration number of 4.23.

Table 4
Multivariate regression analysis for tumor related survival (Cox proportional hazard model).

Category	Hazard ratio	95% Confidence interval	*P*-value
Overall survival			
Age (≤65 years vs. >65 years)	1.01	0.97–1.04	0.694
Gender (female vs. male)	1.54	0.72–3.31	0.264
*p*T1 + 2 vs. *p*T3+4	0.85	0.44–1.96	0.846
*p*N0 vs. *p*N1	3.76	1.56–9.07	0.003
G1 + 2 vs. G3 + 4	1.33	0.69–2.59	0.395
Loss on chromosome 4*q* vs. no loss on chromosome 4*q*	2.94	1.14–7.58	0.026
Recurrence			
Age (≤65 years vs. >65 years)	0.99	0.96–1.03	0.694
Gender (female vs. male)	2.05	0.95–4.42	0.067
*p*T1 + 2 vs. *p*T3 + 4	0.72	0.34–1.51	0.846
*p*N0 vs. *p*N1	2.21	1.01–4.85	0.003
G1 + 2 vs. G3 + 4	0.82	0.41–1.62	0.566
Loss on chromosome 4*q* vs. no loss on chromosome 4*q*	3.25	1.19–8.82	0.021

In order to analyze a more homogeneous tumor collective we selected 52 histologically verified, curatively (*R*0, according to our institutional histo-pathological work-up and prior to the Leeds Protocol) resected PDAC, representing the so far largest CGH data set available in this histological setting. A major obstacle harvesting PDAC tissue of high tumor cellularity as required for comparative genetic methods is the strong desmoplastic reaction observed in a majority of PDAC. Frequently, only a few tumor cells are surrounded by massive amounts of fibrous tissue. This may significantly reduce the sensitivity of CGH analysis due to normal-cell contamination. Therefore, we used laser-assisted micro-dissection for the isolation of PDAC cells and implemented SCOMP to amplify the genome of the few dissected malignant cells. Studies without micro-dissection tend to have lower mean aberration numbers and may also contain samples without any structural imbalances. In contrast, we observed chromosomal aberrations in all tumor specimens analyzed including a high mean aberration number reflecting the high degree of chromosomal instability of PDAC. The most frequent gains were located on 2*q*, 8*q*, 4*q*, 3*q*, 1*q* and 13*q* and the most frequent losses on 17*p*, 18*q*, 19*p*, 6*q*, 8*p*, 9*p*, 22*q* and 1*p*. These findings were generally in line with previous studies performed on tissue specimens (Table 5). CGH studies performed on PDAC cell lines tend to show most of the alterations of primary tumor tissue with a slightly different distribution as well as additional frequent gains on 5*p*, 7*p*, and 20*q* and frequent losses on 17*q*, and 21*q* [12,42–44]. This deviation may be due to chromosomal instability in combination with long-term culture of cell lines resulting in genetic changes that are not observed in the primary tumor sample.

Although some of the loci mentioned above contain tumor suppressor genes or oncogenes presumably important for PDAC progression, the role of most copy number alterations is poorly

Table 5
Published CGH data for pancreatic adenocarcinoma. Studies of established pancreatic cancer cell lines were excluded.

CGH-Method	Patients (n)	Material	UICC[a] I	UICC II	UICC III	UICC IV	Frequent gains	Frequent losses	Prognostic information	Samples w/o aberrations (*n*)	Micro-dissection	WGA	Year	Author	Reference
mCGH	27[b]	ff	6	9	1	4	16*p*, 20*q*, 22*q*, 17*q*, 7*q*, 8*q*	9*p*, 13*q*, 18*q*	no	4	no	no	1996	Solinas-Toldo	[45]
mCGH	6	ff	0	1	1	4	7*p*, 8*q*, 11*q*, 20*q*[d]	1, 3*p*, 6*q*, 8*p*, 9*p*, 11*p*, 13*q*, 17*p*, 18*q*, 19, 20*p*, 21[d]	no	na	no	no	1997	Fukushige	[42]
mCGH	13	ff	na	na	na	na	7*p*, 8*q*, 5*p*, 5*q*, 11*p*, 12*p*, 18*q*	18*p*, 18*q*, 6*q*, 17*p*	no	7	no	no	1997	Mahlamäki	[12]
mCGH	33	ff/FFPE	8	19	2	4	5*p*, 8*q*, 12*p*, 19*q*, 20*q*	18*q*, 10*q*, 8*p*, 13*q*	no	11	no	no	2000	Schleger	[15]
mCGH	20	ff	0	1	5	14	8*q*, 3*q*, 20*q* 7*p*, 1*q*, 13*q*	17*p*, 9*p*, 18*q*, 8*p*, 6*q*, 6*p*, 9*q*, 22*q*	no	0	M	DOP	2002	Harada	[46]
mCGH	27	ff	4	11	7	5	8*q*, 7*q*, 1*q*	9*p*, 17*p*, 4*q*, 6*p*	no	0	no	no	2003	Lin	[47]
aCGH	5	ff	0	4	0	1	7*p*, 8*q*, 11*q*, 20*q*, 7*q*, 12*q*, 17*q*	na	no	0	no	no	2004	Holzmann	[48]
mCGH	15	Smear	0	5	6	4	5*p*, 8*q*, 20*q*, 1*q*, 7*p*, 12*p*	9*p*, 18*q*, 19*p*, 8*p*	no	0	L	DOP	2005	Kitoh	[49]
aCGH	17	Xeno	na	na	na	na	7, 8*q*, 11*p*, 14*q*, 20[d]	1*p*, 3*p*, 4*q*, 5*q*, 6, 8*p*, 9, 13*q*, 15*q*, 16*p*, 17*p*, 18*q*, 19, 20, 21, 22[d]	no	0	no	no	2005	Nowak	[50]
aCGH	23	ff	0	1	4	18	1*q*, 2, 3*q*, 5, 7, 8*q*, 12*p*[d]	1*p*, 4*p*, 6, 8*p*, 9, 17*p*, 18*q*, 21*q*, 22*q*[d]	no	0	M	no	2007	Harada	[51]
mCGH	33	FFPE	5	8	15	5	8*q*, 13*q*, 18*p*, 3*q*, 9*p*, 12*p*	1*p*, 22, 19, 17*p*, 18*q*, 8*p*	yes	5	M	no	2007	Schleicher	[41]
aCGH	27[c]	ff	0	1	3	16	8*q*, 1*q*, 2, 3, 5, 7*p*, 11, 14*q*, 17*q*	9*p*, 18*q*, 6, 1*p*, 13, 17*p*, 3*p*, 14*q*	no	0	M	no	2008	Harada	[13]
aCGH	23	ff	0	1	4	18	7*q*, 12*p*, 19*q*, 6*q*, 7*p*, 8*p*, 5*q*	9*p*, 18*q*, 1*p*, 17*p*, 6*q*, 8*p*, 3*p*, 12*q* Xp	no	1	M	no	2009	Harada	[14]

UICC = Union Internationale Contre le Cancer; w/o = without; WGA = whole genome amplification; mCGH = metaphase CGH; aCGH = array CGH; na = information not available; ff = fresh-frozen tissue; FFPE = formalin-fixed and paraffin-embedded tissue; Xeno = direct xenograft of primary tumor tissue without long-term culture; Smear = smear from a fresh biopsy; M = manual microdissection; L = laser-assisted microdissection; DOP = degenerated oligonucleotide PCR.

[a] As provided in the publication.

[b] All cases were T1-2 tumors, N-category not available for all cases.

[c] UICC stage in seven cases not known.

[d] Not in the order of frequency.

understood. None of the most common alterations (i. e. >30%) in our series was of prognostic significance, but the very high frequency suggests relevance for disease progression. The most frequent aberration of the investigated tumors was deletion of 17*p*, suggesting a loss of the tumor suppressor gene *TP53* (17*p*13). Chromosome locus 8*q*24 was most frequently gained in our series and this alteration potentially represents amplifications of the *MYC* oncogene. However, since the resolution of metaphase CGH used in this screening study is only 5–10 Mbp it is idle to speculate about the affected genes within the identified large alterations. This needs to be scrutinized with high-resolution techniques such as oligonucleotide array-CGH [14], or digital karyotyping with 2nd-generation sequencing.

In conclusion, our data suggests that the frequent alterations at chromosome 8*q*, 9*p*, 17*p* and 18*q* as well as deletions on chromosome 4*q* have an effect on the malignant potential of clinically localized PDAC. Our comprehensive genomic data provide a basis to guide studies using high-resolution methods, such as array-CGH, to uncover the genes located within the chromosomal alterations identified in our mCGH investigation.

References

[1] Bardeesy N, DePinho RA. Pancreatic cancer biology and genetics. Nat Rev Cancer 2002;2:897–909.

[2] Li D, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. Lancet 2004;363:1049–57.

[3] Büchler MW, Kleeff J, Friess H. Surgical treatment of pancreatic cancer. J Am Coll Surg 2007;205:S81–86.

[4] Gaedcke J, Gunawan B, Grade M, Szöke R, Liersch T, Becker H, et al. The mesopancreas is the primary site for *r*1 resection in pancreatic head cancer: relevance for clinical trials. Langenbecks Arch Surg 2010;395:451–8.

[5] Esposito I, Kleeff J, Bergmann F, Reiser C, Herpel E, Friess H, et al. Most pancreatic cancer resections are *r*1 resections. Ann Surg Oncol 2008;15:1651–60.

[6] Verbeke CS, Leitch D, Menon KV, McMahon MJ, Guillou PJ, Anthoney A. Redefining the *r*1 resection in pancreatic cancer. Br J Surg 2006;93:1232–7.

[7] Katz MHG, Hwang R, Fleming JB, Evans DB. Tumor-node-metastasis staging of pancreatic adenocarcinoma. CA Cancer J Clin 2008;58:111–25.

[8] Katz MH, Wolff R, Crane CH, Varadhachary G, Javle M, Lin E, et al. Survival and quality of life of patients with resected pancreatic adenocarcinoma treated with adjuvant interferon-based chemoradiation: a phase ii trial. Ann Surg Oncol; 2011.

[9] Moureau-Zabotto L, Phelip JM, Afchain P, Mineur L, Andre T, Vendrely V, et al. Concomitant administration of weekly oxaliplatin, fluorouracil continuous infusion, and radiotherapy after 2 months of gemcitabine and oxaliplatin induction in patients with locally advanced pancreatic cancer: a groupe coordinateur multidisciplinaire en oncologie phase ii study. J Clin Oncol 2008;26:1080–5.

[10] Crane CH, Varadhachary GR, Yordy JS, Staerkel GA, Javle MM, Safran H, et al. Phase ii trial of cetuximab, gemcitabine, and oxaliplatin followed by chemoradiation with cetuximab for locally advanced (t4) pancreatic adenocarcinoma: correlation of smad4(dpc4) immunostaining with pattern of disease progression. J Clin Oncol 2011;29:3037–43.

[11] Gorunova L, Höglund M, Andrén-Sandberg A, Dawiskiba S, Jin Y, Mitelman F, et al. Cytogenetic analysis of pancreatic carcinomas: intratumor heterogeneity and nonrandom pattern of chromosome aberrations. Genes Chromosomes Cancer 1998;23:81–99.

[12] Mahlamäki EH, Bärlund M, Tanner M, Gorunova L, Höglund M, Karhu R, et al. Frequent amplification of 8*q*24, 11*q*, 17*q*, and 20*q*-specific genes in pancreatic cancer. Genes Chromosomes Cancer 2002;35:353–8.

[13] Harada T, Chelala C, Bhakta V, Chaplin T, Caulee K, Baril P, et al. Genome-wide DNA copy number analysis in pancreatic cancer using high-density single nucleotide polymorphism arrays. Oncogene 2008;27:1951–60.

[14] Harada T, Chelala C, Crnogorac-Jurcevic T, Lemoine NR. Genome-wide analysis of pancreatic cancer using microarray-based techniques. Pancreatology 2009;9:13–24.

[15] Schleger C, Arens N, Zentgraf H, Bleyl U, Verbeke C. Identification of frequent chromosomal aberrations in ductal adenocarcinoma of the pancreas by comparative genomic hybridization (CGH). J Pathol 2000;191:27–32.

[16] Bashyam MD, Bair R, Kim YH, Wang P, Hernandez-Boussard T, Karikari CA, et al. Array-based comparative genomic hybridization identifies localized DNA amplifications and homozygous deletions in pancreatic cancer. Neoplasia 2005;7:556–62.

[17] Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. Nature 1998;396:643–9.

[18] Pino MS, Chung DC. The chromosomal instability pathway in colon cancer. Gastroenterology 2010;138:2059–72.

[19] Artandi SE, Chang S, Lee SL, Alson S, Gottlieb GJ, Chin L, et al. Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. Nature 2000;406:641–5.
[20] DePinho RA. The age of cancer. Nature 2000;408:248–54.
[21] Maser RS, DePinho RA. Connecting chromosomes, crisis, and cancer. Science 2002;297:565–9.
[22] van Heek NT, Meeker AK, Kern SE, Yeo CJ, Lillemoe KD, Cameron JL, et al. Telomere shortening is nearly universal in pancreatic intraepithelial neoplasia. Am J Pathol 2002;161:1541–7.
[23] Shono M, Sato N, Mizumoto K, Maehara N, Nakamura M, Nagai E, et al. Stepwise progression of centrosome defects associated with local tumor growth and metastatic process of human pancreatic carcinoma cells transplanted orthotopically into nude mice. Lab Invest 2001;81:945–52.
[24] Sato N, Mizumoto K, Nakamura M, Maehara N, Minamishima YA, Nishio S, et al. Correlation between centrosome abnormalities and chromosomal instability in human pancreatic cancer cells. Cancer Genet Cytogenet 2001; 126:13–9.
[25] Stoecklein NH, Luebke AM, Erbersdobler A, Knoefel WT, Schraut W, Verde PE, et al. Copy number of chromosome 17 but not her2 amplification predicts clinical outcome of patients with pancreatic ductal adenocarcinoma. J Clin Oncol 2004;22:4737–45.
[26] Linder S, Falkmer U, Hagmar T, Blåsjö M, Sundelin P, von Rosen A. Prognostic significance of DNA ploidy in pancreatic carcinoma. Pancreas 1994;9:764–72.
[27] Weger AR, Graf AH, Askensten U, Schwab G, Bodner E, Auer G, et al. Ploidy as prognostic determinant in pancreatic cancer. Lancet 1987;2:1031.
[28] Stoecklein NH, Erbersdobler A, Schmidt-Kittler O, Diebold J, Schardt JA, Izbicki JR, et al. Scomp is superior to degenerated oligonucleotide primed-polymerase chain reaction for global amplification of minute amounts of DNA from microdissected archival tissue samples. Am J Pathol 2002;161: 43–51.
[29] Pirker C, Raidl M, Steiner E, Elbling L, Holzmann K, Spiegl-Kreinecker S, et al. Whole genome amplification for cgh analysis: linker-adapter pcr as the method of choice for difficult and limited samples. Cytometry A 2004;61: 26–34.
[30] Sobin LHW, Wittekind C. Tnm classification of malignant tumors. UICC. 6th ed. John Wiley & Sons; 2002.
[31] du Manoir S, Schröck E, Bentz M, Speicher MR, Joos S, Ried T, et al. Quantitative analysis of comparative genomic hybridization. Cytometry 1995;19: 27–41.
[32] R, Developement, Core, Team: R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2004.
[33] Baudis M, Cleary ML. Progenetix.Net: an online repository for molecular cytogenetic aberration data. Bioinformatics 2001;17:1228–9.
[34] Karhu R, Kahkonen M, Kuukasjarvi T, Pennanen S, Tirkkonen M, Kallioniemi O. Quality control of cgh: impact of metaphase chromosomes and the dynamic range of hybridization. Cytometry 1997;28:198–205.
[35] Kallioniemi OP, Kallioniemi A, Piper J, Isola J, Waldman FM, Gray JW, et al. Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. Gene Chromosome Canc 1994;10: 231–43.
[36] Baudis M. Progenetix oncogenomic online resource, http://Www.Progenetix.Net; 2009, 2010.
[37] Lin SC, Chen YJ, Kao SY, Hsu MT, Lin CH, Yang SC, et al. Chromosomal changes in betel-associated oral squamous cell carcinomas and their relationship to clinical parameters. Oral Oncol 2002;38:266–73.
[38] De Angelis PM, Stokke T, Beigi M, Mjåland O, Clausen OP. Prognostic significance of recurrent chromosomal aberrations detected by comparative genomic hybridization in sporadic colorectal cancer. Int J Colorec Dis 2001;16: 38–45.
[39] Arai Y, Honda S, Haruta M, Kasai F, Fujiwara Y, Ohshima J, et al. Genome-wide analysis of allelic imbalances reveals 4*q* deletions as a poor prognostic factor and mdm4 amplification at 1*q*32.1 in hepatoblastoma. Gene Chromosome Canc 2010;49:596–609.
[40] Wrage M, Ruosaari S, Eijk PP, Kaifi JT, Hollmen J, Yekebas EF, et al. Genomic profiles associated with early micrometastasis in lung cancer: relevance of 4*q* deletion. Clin Cancer Res 2009;15:1566–74.
[41] Schleicher C, Poremba C, Wolters H, Schäfer K-L, Senninger N, Colombo-Benkmann M. Gain of chromosome 8*q*: a potential prognostic marker in resectable adenocarcinoma of the pancreas? Ann Surg Oncol 2007;14: 1327–35.
[42] Fukushige, Waldman F, Kimura M. Frequent gain of copy number on the long arm of chromosome 20 in human pancreatic adenocarcinoma. Genes; 1997.
[43] Tirado CA, Sandberg AA, Stone JF. Identification of a novel amplicon at 1q31 in pancreatic cancer cell lines. Cancer Genet Cytogenet 1999;113:110–4.
[44] Ghadimi BM, Schrock E, Walker RL, Wangsa D, Jauho A, Meltzer PS, et al. Specific chromosomal aberrations and amplification of the aib1 nuclear receptor coactivator gene in pancreatic carcinomas. Am J Pathol 1999;154: 525–36.
[45] Solinas-Toldo S, Wallrapp C, Müller-Pillasch F, Bentz M, Gress T, Lichter P. Mapping of chromosomal imbalances in pancreatic carcinoma by comparative genomic hybridization. Cancer Res 1996;56:3803–7.
[46] Harada T, Okita K, Shiraishi K, Kusano N, Kondoh S, Sasaki K. Interglandular cytogenetic heterogeneity detected by comparative genomic hybridization in pancreatic cancer. Cancer Res 2002;62:835–9.
[47] Lin M, Cai D, Luo M. Identification of chromosomal imbalances in pancreatic carcinoma using comparative genomic hybridization. Chin Med J (Engl) 2003; 116:1156–60.
[48] Holzmann K, Kohlhammer H, Schwaenen C, Wessendorf S, Kestler HA, Schwoerer A, et al. 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. Cancer Res 2004;64:4428–33.
[49] Kitoh H, Ryozawa S, Harada T, Kondoh S, Furuya T, Kawauchi S, et al. Comparative genomic hybridization analysis for pancreatic cancer specimens obtained by endoscopic ultrasonography-guided fine-needle aspiration. J Gastroenterol 2005;40:511–7.
[50] Nowak NJ, Gaile D, Conroy JM, McQuaid D, Cowell J, Carter R, et al. Genome-wide aberrations in pancreatic adenocarcinoma. Cancer Genet Cytogenet 2005;161:36–50.
[51] Harada T, Baril P, Gangeswaran R, Kelly G, Chelala C, Bhakta V, et al. Identification of genetic alterations in pancreatic cancer by the combined use of tissue microdissection and array-based comparative genomic hybridisation. Br J Cancer 2007;96:373–82.