Pancreatology 12 (2012) 16–22

Contents lists available at SciVerse ScienceDirect

Pancreatology

journal homepage: www.elsevier.com/locate/pan

Original article

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

A.M. Luebke [a,c,1], M. Baudis [d,1], H. Matthaei [b,1], Y.K. Vashist [a], P.E. Verde [e], S.B. Hosch [a,2], A. Erbersdobler [f,3], C.A. Klein [g], J.R. Izbicki [a], W.T. Knoefel [a,2], N.H. Stoecklein [a,b,2,*]

[a] *Klinik und Poliklinik für Allgemein-, Visceral- und Thoraxchirurgie, Universitätsklinikum Hamburg-Eppendorf, Germany*
[b] *Klinik für Allgemein-, Viszeral- und Kinderchirurgie, Universitätsklinikum Düsseldorf, Germany*
[c] *Institut für Pathologie, Universitätsklinikum Hamburg-Eppendorf, Germany*
[d] *Institute of Molecular Life Sciences, Universität Zürich, Switzerland*
[e] *Koordinierungszentrum für klinische Studien, Universitätsklinikum Düsseldorf, Germany*
[f] *Institut für Pathologie, Universitätsklinikum Rostock, Germany*
[g] *Lehrstuhl für Experimentelle Medizin und Therapieverfahren, Universität Regensburg, Germany*

Keywords:
CGH
PDAC
Pancreatic cancer
4*q* loss
Clinical follow-up
Prognosis

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.

* Corresponding author. Klinik für Allgemein-, Viszeral-, und Kinderchirurgie, Universitätsklinikum Düsseldorf, Moorenstr. 5, D-40225 Düsseldorf, Germany. Tel.: +49 (0)211 8104109; fax: +49 (0)211 8104494.
E-mail address: nikolas.stoecklein@uni-duesseldorf.de (N.H. Stoecklein).
[1] Contributed equally to this study.
[2] Present address: Klinik für Allgemein-, Viszeral- und Kinderchirurgie, Universitätsklinikum Düsseldorf, Germany.
[3] Present address: Institut für Pathologie, Universitätsklinikum Rostock, Germany.

1424-3903/$ – see front matter
doi:10.1016/j.pan.2011.11.001

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.