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Invasive Ductal Carcinoma of the Pancreas, Measuring 2 cm or Less in Greatest Dimension：A Clinicopathologic Study with an Analysis of Aberrant Chromosomal Regions Using CGH

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Abstract：We examined 14 cases of invasive ductal carcinoma (IDC) of the pancreas measuring ≤2 cm in greatest dimension (pTS1) to clarify their clinicopathologic features, and analyzed chromosomal genetic alterations using comparative genomic hybridization (CGH). Patients' ages ranged from 51–80 years (average 64.8 years, median 66 years), with eight males and six females. A majority of eight cases showed epigastralgia or back pain, but four cases were asymptomatic. Eight tumors arose in the body and six in the head of the pancreas. For histological grades of tubular adenocarcinoma, there were five well differentiated, four moderately differentiated, and five poorly differentiated types. Nodal metastasis was found in five cases. Even in pTS1–IDC of the pancreas, the stage of cancer progression was highly advanced with seven cases (50.0%) in Stage II or over. For the prognosis, five of the 14 patients died, of which four (28.6%) resulted from hepatic metastasis. For CGH analysis, we detected highly frequent losses on chromosome arms 1p, 9q, 12q, 16p, 16q, 17p, 17q, 19p, 19q, 20q, and 22q, and frequent gains on chromosome arms 4q, 6q, and Xq. Losses of 1p34–pter, 17p12–pter, and 22q12 were observed in five cases suffering a nodal metastasis. Losses of 1p35–pter and 9q33–qter were detected in four cases that died of postoperative liver metastasis. These altered chromosomal regions may contain the genes involved in the carcinogenesis, progression, and metastatic properties of pTS1–IDC of the pancreas.

Key wards：**Pancreas, Invasive ductal carcinoma, pTS1, CGH**

Introduction

Early detection of invasive ductal carcinoma (IDC) of the pancreas remains a difficult process, and when compared with other digestive system cancers, is regarded as having an unfavorable prognosis. According to statistics from the Japan Pancreas Society, for the 1991–2000 period, the pancreatic resection rate for IDC cases was relatively low (40%), with an accompanying five year survival rate of 13.4% following surgery.[1)] However, Egawa et al.[2)] found that even in stage IVa advanced-stage cancer, excising tumors that were 2 cm or less in maximum diameter (pTS1≤2 cm) improved the five year survival rate to 22.3%, which was more favorable than the corresponding rate for pancreatic cancers larger than 2 cm in size. Thus in any future attempt to improve prognosis for pancreatic cancer, the early identification of even the smallest of pancreatic cancers is important. To achieve this, elucidation of the clinicopathologic features of pTS1–IDC of the pancreas is essential.

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Existing molecular－biological research has been successful in unraveling the contribution of genetic disease to the onset and development of human cancer, with such studies indicating that accumulation of abnormalities in various oncogenes and tumor suppressor genes is a contributing factor to cancer development and progression.[3)4)] To date, among the studies concerning human pancreatic cancer, a wide number of genetic abnormalities have been reported in genes such as K－ras (12p12),[5)6)] p53 (17p13.1),[6)] Smad4/DPC4 (18q21.1),[7)－13)] $p16^{INK4a}$ /MTS1/CDKN2 (9p21),[14)] p21 (waf1/cip1),[15)] APC (5q21–q22),[16)] DCC (18q21.3),[17)] Rb (13q14.1–q14.2),[18)] BRCA–2 (13q12–13),[19)] and AKT2 (19q13).[20)] Thus broad–ranging analysis of chromosomes and genes is now indispensable in efforts to elucidate the mechanisms underlying carcinogenesis and progression of IDC of the pancreas.

The comparative genomic hybridization method (CGH), developed by Kallioniemi et al.,[21)] is currently used for genetic analysis of various tumors. The advantages of this method relate to its ability to allow simultaneous detection of changes in the relevant tumors' DNA copy number through performing a single hybridization process, rather than the simultaneous use of conventional multiple–base sequence–specific probes. Despite some discrepancies in frequency, examining pancreatic cancer primary tumors with CGH analysis has successfully identified multiple diverse aberrant chromosomal regions. Although this is thought to reflect possible multi－step changes in genes,[22)] this may also show the detection of secondary abnormalities or additional abnormalities[23)] arising from the onset and development process of tumors. However, small pancreatic cancers maintain only limited secondary abnormalities or additional abnormalities, and the detection of aberrant genes is thought to be able to take place at an earlier invasive stage.

Here we report 14 cases with pancreatic invasive ductal carcinoma of 2 cm or less in microscopic tumor size (pTS1–IDC). The tumors were examined clinicopathologically, then the aberrant genetic regions analyzed by the CGH method combined with a laser capture microdissection (LCM) system.

Materials and Methods

Tissues

During a 12–year–8–month period from April 1991 to December 2004, a total number of 199 cases with conventional IDC of the pancreas were examined histologically at the Department of Pathology, Faculty of Medicine, Fukuoka University, Fukuoka, Japan. From the 199 IDCs, 14 (7.0%) were microscopically 2cm or less in greatest diameter (pTS1≤2 cm), and were used in the present study.

Histopathological analysis

To avoid autolytic changes of the tissues due to pancreatic juice, all pancreata were fixed by tubing and were infused with 10% formalin through the duodenal papilla and/or the pancreatic resection stump of the main pancreatic duct immediately after their surgical resection. After fixation, pancreatography was performed using 80 to 100% barium, and a cut made on the main lesion with fluoroscopic guidance. The pancreata were then sliced in a serial fashion at approximately 3–5 mm intervals in the head–to–tail direction. Photocopies and color photographs of all slices were taken and each slice was examined macroscopically. Tissue blocks were taken from the whole pancreatic specimen and routinely processed to paraffin sections. All sections (3 μm thick) were stained with hematoxylin and eosin and examined. Thus a three－dimensional reconstruction of the lesion was determined and the tumor size was calculated microscopically.[24)25)]

Microdissection and sample preparation

All tumor specimens were selectively collected from paraffin－embedded tissue sections (8 μm thick) by using a laser capture microdissection apparatus (PixCell® IIe LCM ; Arcturus, CA, USA). Tissue sections were stained with hematoxylin－eosin to identify cancerous regions. Approximately 300 to 500[26)] target tumor cells were microdissected and digested into 10 μl of the proteinase K (Sigma –Aldrich) solution (40 mg/ml 1x Thermo Sequenase Reaction buffer ; Amersham Life Science, Cleveland, OH, USA) for 18 hours at 42℃ followed by

heating to 95℃ for 5min to inactivate the proteinase K. This proteinase K solution was directly used for the template of degenerate oligonucleotide primed-polymerase chain reaction (DOP-PCR) amplification.

DOP-PCR

DOP-PCR was performed in two steps according to a previously published protocol by Qiang Huang et al.[27] with some modifications. Briefly, the first step was performed in a 5.0μl final reaction mixture consisted of 3.1μl of the template, 0.5μl of 10x Thermo Sequenase Reaction buffer, 0.5μl of 4U/μl Thermo Sequenase DNA polymerase(Amersham Life Science, Cleveland, OH), 0.4μl of 2.5mM dNTP (PCR Nucleotide Mix ; Roche), and 0.4μl of 10mM DOP primer (5'-CCG ACT CGA GNN NNN NAT GTG G-3'). The amplification profile was an initial denaturation of 4min at 95℃, followed by 8 cycles of 2min at 95℃, 1min at 25℃, 3min ramp from 25 to 74℃, 3min at 74℃, and a final extension of 10 min at 74℃. The second step was performed in a final reaction of 25.0μl consisting of the first step PCR product, 2.0μl of 10x PCR buffer, 0.5μl of Ampli Taq DNA polymerase LD (Applied Biosystems, USA [0]), 0.7μl of 10mM dNTP (10mM dATP 0.2μl, 10mM dGTP 0.2μl, 10mM dTTP 0.1μl, 10mM dCTP 0.2μl), 1.0μl of 1mM fluorescein-12-dUTP (Roche Diagnostics, Mannheim, Germany), 3.0μl of 10mM DOP primer, and 7.8μl of distilled water. The amplification profile was an initial denaturation of 4min at 95℃, followed by 37 cycles of 1min at 95℃, 1 min at 56℃, 3min at 72℃, and a final extension of 10 min at 72℃. The 2nd step of DOP-PCR product was confirmed by agarose gel electrophoresis as a smear of fragments between 300bp and 500bp in size.

CGH and digital image analysis

CGH was performed essentially as described by Kallioniemi et al.[21] Nineteen microliters of the 2nd step of DOP-PCR product and 1μl of reference DNA (SpectrumRed™ Direct-Labeled Normal Female or Male Total Human Genomic DNA ; Vysis, Inc. Downers Grove, IL, USA) were hybridized together with 10μl of Human Cot-1 DNA (Vysis, Inc.) to normal denatured metaphase chromosomes (Normal 46, XY, Metaphase CGH Target Slide; Vysis, Inc.) for three days at 37℃, and counter stained with 4, 6-Diamidino-2-Phenylinode(DAPI; Sigma-Aldrich).

The hybridization was analyzed by using the digital image analysis system (ISIS ; Carl Zeiss Vision, Oberkochen, Germany) based on an integrated high-sensitivity monochrome charge-coupled device (CCD) camera and automated CGH analysis software (MetaSystems, Altlussheim, Germany). For each sample, three color images (green for tumor DNA, red for reference DNA, and blue for counterstaining) were captured from at least seven metaphases and processed for more than 10 high quality chromosomes.

Chromosomal regions with a green : red ratio of >1.2 were considered as gains (overrepresented), whereas regions with ratios of <0.8 were considered as losses (underrepresented). These cut-off values were based on series of normal hybridization experiments. The heterochromatic regions in chromosomes and the Y chromosome were excluded from the analyses because of suppression of hybridization with Cot-1 DNA in these regions. As the ratio of fluorescence intensity can be unstable, telomeric regions were also excluded from the analyses.

Results

Clinical findings

The clinical features of 14 cases with pTS1-IDC are shown in Table 1. The age of patients ranged from 51 to 80 years (average age 64.8 years, median value 66 years), with a majority of males to females (8 : 6). A majority of eight cases (80.0% of existing symptoms) showed abdominal pain and back pain. One case complained of diabetic symptoms, while the other case had jaundice. Four out of these 10 cases were further found to have an episode of acute pancreatitis. A total of four cases (28.6%) reported no obvious symptoms, and three of them (75.0%) were discovered by abdominal ultrasonography (US) during health checkups. From the results of the diagnostic image, 12 of the 14 cases (85.7%) had the initial finding of the disease locus detected by routine abdominal US. The remaining two cases were found to have constriction

of the main pancreatic duct through endoscopic retrograde pancreatography, and dilation of the main pancreatic duct through abdominal computed tomography.

From the results of the balloon endoscopic retrograde pancreatography－compression study,[28] all of the 14 cases were diagnosed as having a conventional IDC and subsequently underwent surgery (Table 1). The postoperative follow－up examination ran for a period from 2.6 years to a maximum of 15.3 years (average 5.0 years, median 4.0 years). Two cases were subsequently found to have survived for more than five years. A total of five of the 14 cases died, of whom four died from hepatic metastasis with two cases dying within one year of surgery, of which one case was found to be at JPS Stage I (UICC Stage I a). The remaining one case died of another disease (primary squamous cell carcinoma of the lung).

Histopathological findings

A summary of the histopathological findings is shown in Table 1. The location of the tumor was identified as being at the head of the pancreas in six cases, and in the pancreas body in the other eight cases. All cases were found to have a poorly demarcated infiltrative tumor, of whitish gray color, accompanied by stromal fibrosis. Histologically, the tumor size was measured by a three dimensional recreation, with the maximum diameter being 0.8 to 2 cm (average 1.7 cm). In predominant grades of differentiation, five cases (35.7%) were determined to have a well differentiated tubular adenocarcinoma (Figure 1a), while there were four cases (28.6%) with a moderately differentiated type (Figure 1b), and a further five cases (35.7%) with a poorly differentiated type (Figure 1c). The adjacent acinar lobules were significantly atrophic or absent, with marked stromal fibrosis and a chronic inflammatory infiltration. Lymphatic or venous permeation by the carcinoma was found in 12 cases (85.7%) or 10 cases (71.4%), respectively. Neural invasion by the carcinoma was also found in 12 cases (85.7%). Three cases (21.4%) were found to have an invasion of the intrapancreatic bile duct (Figure 2a), with two cases (14.3%) having duodenal invasion (Figure 2b), while three cases (21.4%) had peripancreatic retroperitoneal invasion (Figure 2c). One case had an invasion of the adventitia of the splenic vein (Figure 2d). Metastasis to the regional lymph nodes was observed in five cases (35.7%). The stage of cancer progression (JPS Stage) was Stage I in seven cases (53.3%), Stage II in one case (7.1%), Stage III in four cases (28.6%), and Stage IVa in two cases (14.3%). Surprisingly, even in cases with pTS1–IDC, the stage of cancer progression was seen as highly advanced with seven cases (50.0%) in Stage II or over.

Table 1. Clinicopathologic features of 14 cases with pTS1 invasive ductal carcinoma of the pancreas.

Case No.	Age/sex	Location	Tumor size (cm)	Grade* of differentiation	JPS fStage	Operative procedure	Outcome (year)
1	73/F	Head	1.8	moderately	I	PPPD	0.5, died of liver metastasis
2	53/M	Body	1.5	moderately	I	DP＋Sp	15.3, alive
3	61/F	Head	1.5	moderately	IVa	PPPD	13.8, alive
4	62/M	Head	2.0	well	I	PD	4.2, died of other disease†
5	63/F	Head	2.0	poorly	III	PPPD	0.6, died of liver metastasis
6	72/M	Head	0.8	well	I	PPPD	6.8, alive
7	66/M	Body	1.8	well	II	PPPD	1.6, died of liver metastasis
8	51/F	Body	1.9	poorly	IVa	DP＋Sp	3.5, alive
9	60/F	Head	2.0	poorly	III	PPPD	5.5, alive
10	80/F	Body	1.6	well	III	DP＋Sp	2.7, died of liver metastasis
11	67/M	Body	2.0	moderately	III	DP＋Sp	5.3, alive
12	61/M	Body	1.6	well	I	DP＋Sp	4.3, alive
13	68/M	Body	1.5	poorly	I	TP	3.8, alive
14	70/M	Body	1.6	poorly	I	DP＋Sp	2.6, alive

* The predominant grade of tubular adenocarcinoma is classified into three types according to the tubular formation: well, well differentiated type; moderately, moderately differentiated type; poorly, poorly differentiated type; JPS, Japan Pancreas Society histological classification System (5th edition); fStage, final stage. PD, pancreaticoduodenectomy; PPPD, pylorus－reserving pancreaticoduodenectomy; DP, distal pancreatectomy; Sp, splenectomy; TP, total pancreatectomy; †, Died of primary squamous cell carcinoma of the lung.

Relation of microscopic tumor size to prognosis

The resection rate for the patients with a conventional IDC of the pancreas was 41.5% in our surgery. There was no significant difference between the one-year survival rates in terms of prognosis for both "pTS1" and "pTS2 to pTS4" groups. In examining the three- and five-year survival rates, however, in contrast to the favorable prognosis of 67.3% and 56.1%, respectively, for the patients with "pTS1", the comparable rates for cases with "pTS2 to pTS4" were 19.2% and 11.2%, respectively, indicating an unfavorable prognosis (postoperative cumulative survival rate by Kaplan-Meier method, P=0.005, Mann-Whitney U test).

Analysis of aberrant chromosomal regions using CGH

CGH analysis was performed on 14 cases identified as having pTS1-IDC. All of these 14 cases were successful in analysis of the chromosomal region. The CGH analysis results for all 14 cases are shown in Table 2 and Figure 3. In CGH analysis of chromosomal regions of the 14 cases with pTS1-IDC, we detected a wide range of abnormal alterations. We detected copy numbers losses of DNA sequences on chromosome regions1p, 1q, 2p, 2q, 5q, 6p, 6q, 8p, 8q, 9q, 10q, 11q, 12p, 12q, 13q, 14q, 15q, 16p, 16q, 17p, 17q, 18p, 18q, 19p, 19q, 20q, 21q, 22q, Xp, and Xq regions and gains of 2q, 3q, 4q, 5q, 6q, 13q, Xp, and Xq. Losses were more frequent in comparison with gains. Highly frequent losses over 50% were confirmed on chromosome arms 1p, 9q, 12q, 16p, 16q, 17p, 17q, 19p, 19q, 20q, and 22q. Highly frequent gains over 50% were also observed on chromosome arms 4q, 6q, and Xq. In carrying out CGH analysis, we detected loss of the 17p13.1 chromosome on the p53 gene locus in 12 cases (85.7%), and loss of the 18q21.1 chromosome on the DPC4 gene locus was observed in four cases(28.6%). In comparing clinicopathological findings and the results of CGH analysis, loss of the 1p35-pter and 9q33-qter regions were found in four cases who died of liver metastasis (case numbers 1, 5, 7, and 10). Fur-

Table 2. CGH results of 14 cases with pTS1 invasive ductal carcinoma of the pancreas.

Case No	Chromosomal aberrations	
	Losses	Gains
1	1p34-pter, 9q34-qter, 12q24-qter, 15q22-qter, 16p13-pter, 20q12-qter, 22q11.2-qter	
2	1p32-pter, 9q32-qter, 10q25-qter, 15q21-q25, 16p13-pter, 17p13, 17q21-qter, 19q13.2, 20q12-qter, 22q12.1-qter	6q11-q16, Xp11-p21, Xq
3	1p32-pter, 1q41-qter, 2p24-pter, 6p23-pter, 9q34-qter, 10q25-qter, 12q24-qter, 14q24-qter, 16p12-pter, 16q23-qter, 17p12-pter, 17q21-qter, 19p, 19q, 20p12-pter, 20q12-qter, 21q22.1-qter, 22q11.2-ter	
4	1p33-pter, 1q42-ter, 2p23, 8q24q-ter, 9q34-ter, 10q26-ter, 14q31-qter, 15q22-q24, 16p13-pter, 16q22-qter, 17p12-ter, 17q21-qter, 19p13.2-ter, 19q, 20q, 22q12.1q-ter	4q22-q26, 5q21, 13q21-31, Xp, Xq
5	1p34-pter, 9q34-qter, 12q22-q23, 14q23-q24, 15q21-q24, 17p12-pter, 17q21-qter, 18q21, 20q13.1, 22q11.2-q13.2, Xp22-p11	4q22-q28, 6q11-q22
6	1p34-pter, 5q32-qter, 8p22-p21, 9q31-qter, 10q24-qter, 11q23-qter, 12q24-qter, 15q 22-q25, 16p12-p13, 16q23-qter, 17p12-pter, 17q12-q24, 18q21, 19p, 19q, 20q12-qter, 22q11.2-qter	4q11-31, 6q11-22, 13q14-q22, Xp, Xq
7	1p34-pter, 8q24-qter, 9q33-qter, 10q24-qter, 11q23-qter, 20q12-qter, 21q22.1-qter, 22q11.6-ter, 16p12-pter, 16q22-qter, 17p13-pter, 17q23-qter, 19p13.3-pter, 19q13.1-q13.3, 20p12, 20q12-qter, 22q11.2-q13.2	4q11-q31, 5q21-q22, 6q11-q16, 13q21-q22, Xp11-p21, Xq
8	1p32-pter, 8q24-qter, 9q33-qter, 12q24-qter, 16p11-pter, 17p, 17q, 19p, 19q, 20q12-qter, 22q	3q24-q26, 4q, 5q14-q23, 6q11-q21, Xp21-p11, Xq
9	1p34-pter, 6q25-qter, 8p21-pter, 11q13, 12q24-qter, 14q31-qter, 16p12-pter, 16q23q-ter, 17p13-pter, 17q, 18p11.3-pter, 18q12-qter, 19p, 19q, 20q12-qter, 22q	2q23-q31, 4q11-q28, 13q21-q22, Xq21-q23
10	1p35-p36.1, 9q22-qter, Xp21, Xq26-qter	
11	1p36-pter, 12q24-qter, 16p11-pter, 17p12-pter, 18q21, 19p13.2-pter, 22q12	4p27, 6q11-q23, Xq
12	1p34-pter, 2q36-qter, 6p22, 9q33-qter, 12p12-pter, 12q24-qter, 13q31-q33, 16p11-pter, 16q12-qter, 17p, 17q, 19p, 19q, 20q, 21q, 22q	2q23-q33, 4q11-q31, 5q14-q23,6q11-q21
13	1p34-pter, 16p12-p13, 16q22-qter, 17p13, 19p, 22q, Xp, Xq	4q22-q27, 6q11-q22,
14	1p32-ter, 9q33-qter, 10q25-qter, 11q23-qter, 12q24-qter, 16p12-pter, 16q22-qter, 17p13-ter, 19p13.3-pter, 19q13.1-q13.2, 20q12-qter, 21q22.1-qter, 22q11.2-qter	4q22-q26, Xq11-q25

CGH, comparative genomic hybridization ; Loss, chromosomal regions ratio of >1.2 ; Gain, chromosomal regions ratio of <0.8.

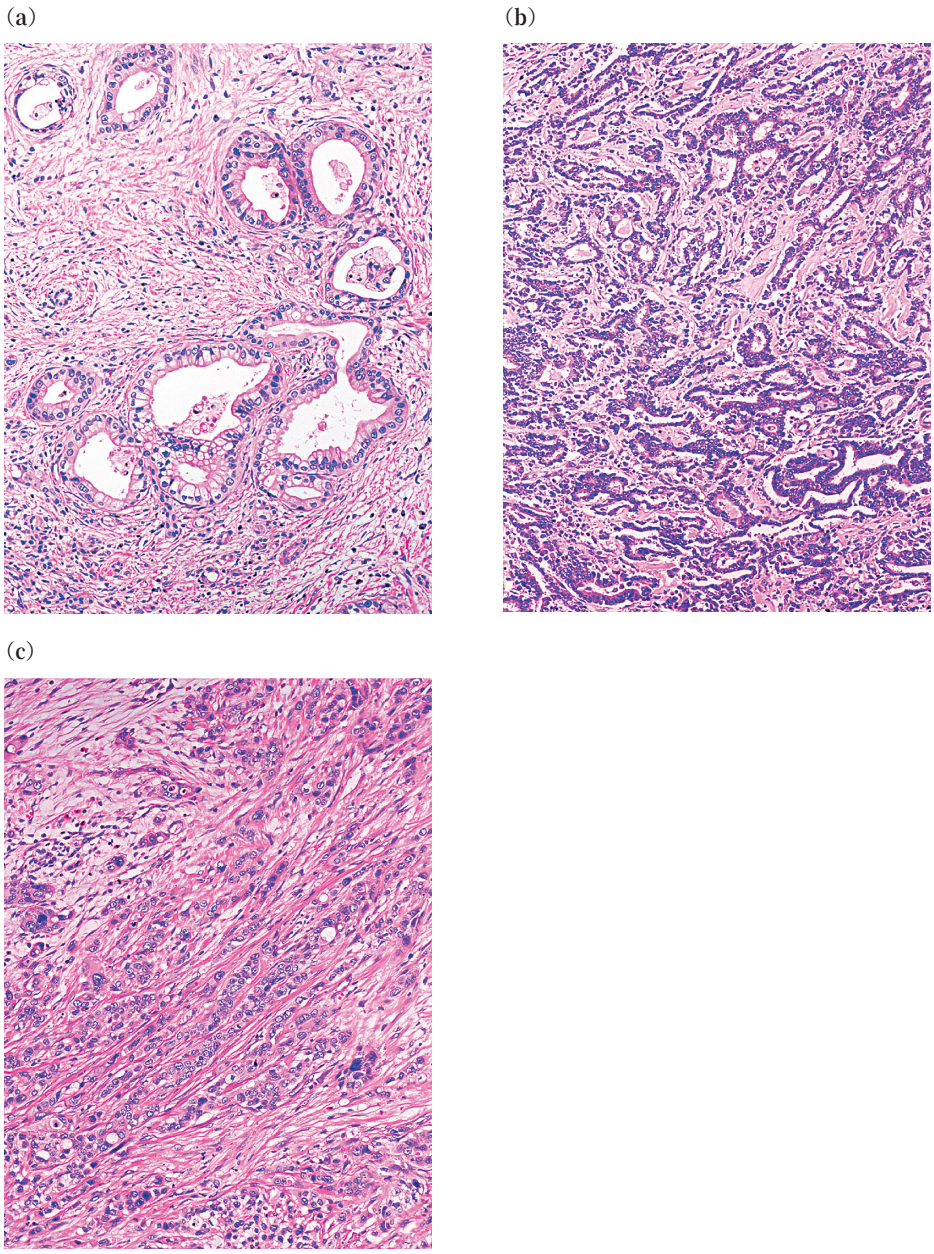

Figure 1. Histologic grade of differentiation of pTS1–IDC of the pancreas.
(a) Well differentiated type of tubular adenocarcinoma showing large, well-formed tubular glands in the fibrous stroma (case number 7). (b) Moderately differentiated type of tubular adenocarcinoma showing smaller irregularly fused tubular structures in the fibrous stroma (case number 1). (c) Poorly differentiated type of tubular adenocarcinoma showing cords, irregular clusters, and single cells with occasional tiny tubules (case number 14). (a–c) Hematoxylin–eosin staining.

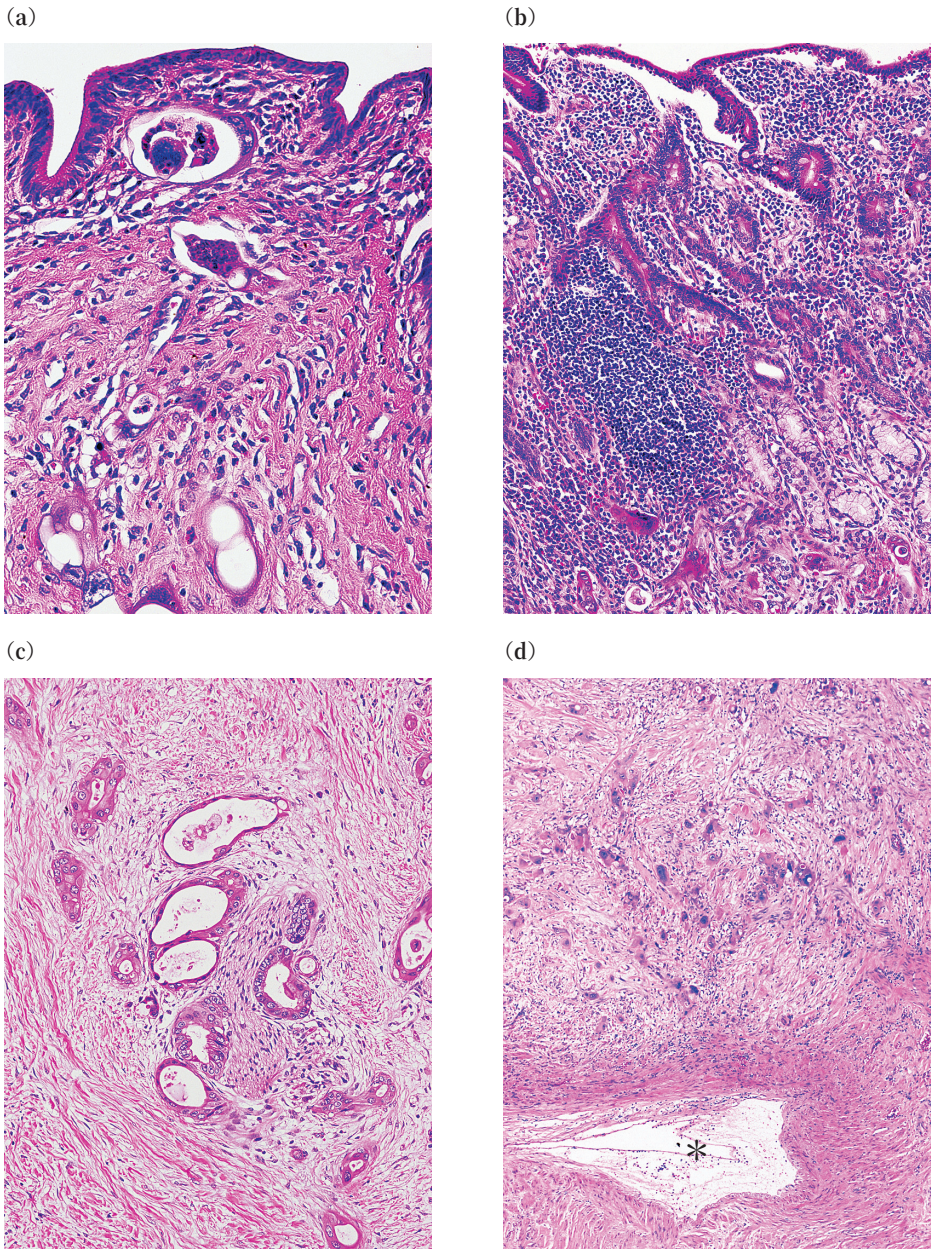

Figure 2. Histopathological findings of pTS1–IDC of the pancreas.
(a) Invasion of the intrapancreatic bile duct (case number 3). The cancer cells infiltrate into the wall of the intrapancreatic bile duct, accompanied by lymphatic permeation by cancer cells. (b) Invasion of the duodenal wall (case number 5). The cancer cells infiltrate into the muscularis propria layers to the mucosa of the duodenum. (c) Neural invasion of the peripancreatic retroperitoneal tissue (case number 11). (d) Invasion of the portal vein (case number 8). The cancer cells infiltrate into the adventitia of the splenic vein (*). (a–d) Hematoxylin–eosin staining.

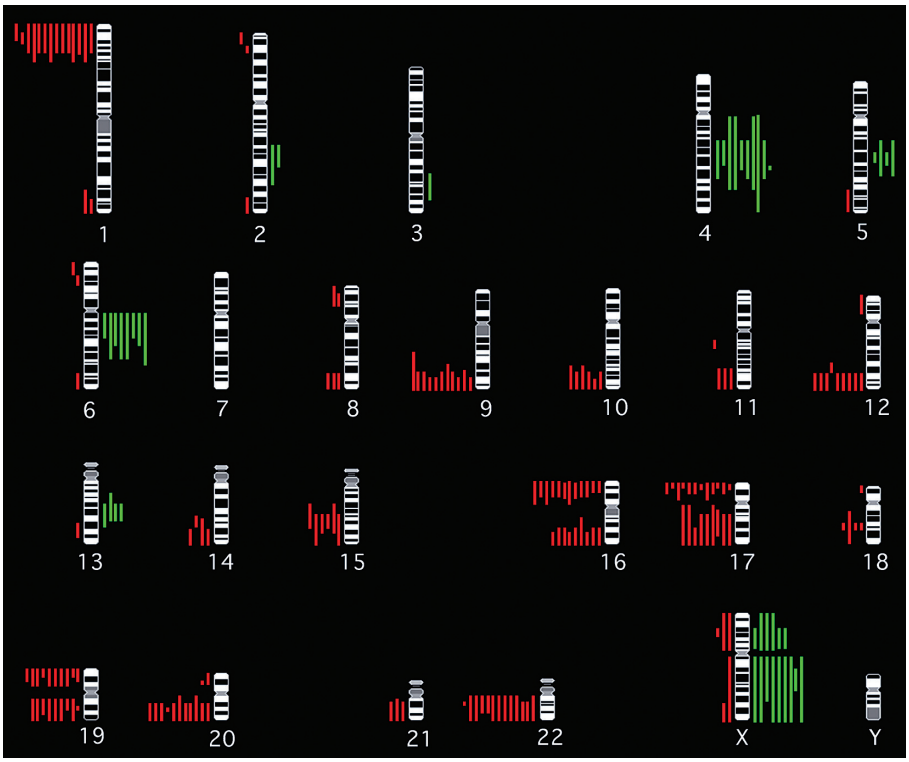

Figure 3. Summary of gains and losses of DNA sequence copy number in primary tumors from 14 cases of pTS1–IDC of the pancreas.
The left side (left) and right side (right) perpendicular lines of the chromosome show the gains and losses of DNA copy numbers in the tumors. Losses are of high frequency in comparison with gains. Highly frequent losses of over 50% are detected on chromosome arms 1p, 9q, 12q, 16p, 16q, 17p, 17q, 19p, 19q, 20q, and 22q. On the other hand, highly frequent gains over 50% are observed on chromosome arms 4q, 6q, and Xq.

Table 3. Comparison of CGH results on invasive ductal carcinoma of the pancreas in the literature.

	Materials (n)	Chromosomal aberrations	
		Losses	Gains
Solinas－Toldo at al.[30]	FR (27)	6q, 9p, 13q	16p, 16q, 17q, 18p, 20p, 20q, 22q
Mahlamaki at al.[34]	FR (24)*	3p, 6q, 9p, 13q, 15q, 17p, 18p, 18q, 21q	3q, 5p, 5q, 6p, 7p, 7q, 8q, 11p, 11q, 12p, 14q, 17q, 18q, 19q, 20p. 20q
Fukushige at al.[35]	FR (18)*	3p, 4q, 6q, 8p, 9p, 18q, 21q, Y	5p, 7p, 7q, 8q, 11q, 14q, 18p, 20p, 20q
Schleger at al.[36]	FR and FX(33)	1p, 3p, 8p, 10q, 13q, 15q, 18q	3q, 7p, 8q, 17q, 20q
Shiraishi at al.[37]	FR (27)	1p, 3p, 6q, 8p, 9p, 12q, 17p, 18q, 19p	1q, 5p, 5q, 7p, 7q, 8q, 12p, 19q, 20q, 20q
Harada at al.[38]	FR (20)	3p, 4q, 6p, 6q, 8p, 9p, 9q, 12q, 14q, 15q, 16p, 17p, 18q, 20q, 21q, 22q, Xp	1q, 2q, 3q, 5p, 5q, 7p, 8q, 11q, 12p, 12q, 13q, 15q, 17q, 20q
Lin at al.[39]	FR (27)	1q, 4p, 4q, 5p, 6p, 7q, 9p, 10p, 10q, 11p, 11q, 13q, 16q, 17p, 18p, 20p, Xq	1q, 2p, 3q, 4p, 4q, 6q, 7q, 8q, 9q, 10q, 11p, 16p, 18p, 20p, 22q, Xq
Current study	FX (14)	1p, 1q, 2p, 2q, 5q, 6p, 6q, 8p, 8q, 9q, 10q, 11q, 12p, 12q, 13q, 14q, 15q, 16p, 16q, 17p, 17q, 18p, 18q, 19p,19q, 20q, 21q, 22q, Xp, Xq	2q, 3q, 4q, 5q, 6q, 13q, Xp, Xq

* Containing materials of cell line ; n, number of samples, FR, fresh－frozen tissue ; FX, formalin－fixed tissue.

thermore, loss of the 1p34－pter, 17p12－pter, and 22q12 regions were common to five cases with nodal metastasis (case numbers 3, 5, 7, 8, and 11).

Discussion

A number of studies have examined the use of CGH analysis for pancreatic cancer, including that by Salina－Toldo et al.[29] The present study, however, is the first to actually use the LCM system on surgically obtained samples of human TS1 pancreatic cancer cells to selectively obtain tumor cells, and to case these with DOP－PCR CGH analysis. We found that even in pTS1–IDC (≤2 cm), it was possible to detect numerous chromosomal aberrant regions in the same way as that used in the CGH system to analyze significantly advanced cancer tumor samples. This is likely a result of the existence of multiple chromosomal and genetic abnormalities in IDC of the pancreas, even in small tumors detected at an early invasive stage. In contrast, although we were able to detect deletion of the 17q and 19q regions in areas with over 50% high frequency of deletion, in CGH analysis carried out to date, there have been no reports concerning the deletion of the 17q and 19q regions in human pancreatic cancer (Table 3). The deletion of the 17q region in human prostrate cancer has been reported,[30] and a number of studies have reported the deletion of the 19q region in oligodendroglial tumors[31] and sporadic breast cancer,[32] although any relationship with the tumorigenesis or progression of these cancers remains unclear.

In the present study, of the 14 cases with pTS1–IDC, four cases who died from hepatic metastasis shared the deletion of the 1p35－pter and 9q33－qter regions. All five cases suffering from lymph node metastasis shared deletion of the 1p34－pter, 17p12－pter, and 22q12 regions. Studies to date have reported the deletion of the 1p, 9q, 17p, and 22q regions in samples from primary pancreatic duct cancer cells.[29) 33)－38)] However, with the exception of deletion of the 17p region containing the p53 gene locus (17p13.1), any relationship with pancreatic cancer remains unclear. Kimura et al.[39] were able to detect a loss of heterozygosity (LOH) in regions 1p and 17p in human pancreatic cancer, although they did not describe this findings' re-lationship to carcinogenesis and progression of pancreatic cancer. Borg et al.[40] compared tumors of 2 cm or less in diameter and over 2 cm in diameter obtained from primary breast cancer cells, and found that in the tumors larger than 2 cm in diameter the deletion of the 1p allele was connected to lymph vessel metastasis. Conventional thinking holds that among isolated and identified suppressor genes, the 9q region contains the PTC and TSC1 genes, with the 17p region containing the p53 gene, and the NF2 gene localized to the 22q region. This was thought to provide evidence of possible association with hepatic metastasis and nodal metastasis in IDCs of the pancreas.

In the present study, through CGH analysis of the 14 cases with pTS1–IDC, 12 cases (85.7%) were found to have a high degree of frequency of deletion of the region including the 17p13.1 chromosome. In excluding results gained from previous studies to date using pancreatic duct cancer cultured cells, these data follow on from a previous study by Harada et al.[34] which demonstrated similar findings in 90% of cases, and showed a high detection incidence of deletion (Table 3). This region contains the localized p53 gene[6] —a cancer suppressor gene— and deletion or mutation of this gene has been detected with a high incidence rate of approximately 50% in human cancer cases. This mutation figure rises to 50 to 75% in cases of human pancreatic cancer.[6]

Through DOP–PCR analysis we found four cases (28.6%) to have deletion of the region including the 18q21.1 chromosome. The 18q21.1 chromosome contains the suppressor gene DPC4(deleted in pancreatic carcinoma, locus 4) identified from pancreatic cancer. The DPC4 gene is a member of the Smad family of signal transduction cascades that belong to the transforming growth factor－β superfamily, and is known to be active in regulating cell growth, differentiation, and induction of apoptosis.[8)－10)] Mutation of the DPC4 gene is thought to be present in 55% of cases of pancreatic cancer.[7] One of the reasons for the difference in the degree of DPC4 mutation in the literature and DOP－PCR CGH analysis is because the CGH method, while detecting DNA copy losses that are larger than 10 to 20 Mb in size, does not allow detection of smaller abnormalities.

In carrying out the first selective selection of tumor cells using the LCM system, and through subsequent analysis using DOP－PCR CGH, we were successful in detecting multiple aberrant chromosomal regions in cases with pTS1–IDC. From these data, we suggest a connection between the deletion of the 1p35–pter and 9q33–qter regions and hepatic metastasis, and deletion of the 1p34－pter, 17p12－pter, and 22q12 regions and nodal metastasis. Taken together, the data obtained from the present study indicate that abnormalities in these genes may also be involved in the carcinogenesis, progression, and metastasis of pTS1–IDC of the pancreas.

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