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The Implication of Cytogenetic Alteration in Pancreatic Ductal Adenocarcinoma and Intraductal Papillary Mucinous Neoplasm Identified by Fluorescence *In Situ* Hybridization and the Potential Usefulness for the Diagnosis

형광동소보합법을 이용한 췌장암 및
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임 창 섭

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Abstract

The Implication of Cytogenetic Alteration in Pancreatic Ductal Adenocarcinoma and Intraductal Papillary Mucinous Neoplasm Identified by Fluorescence *In Situ* Hybridization and the Potential Usefulness for the Diagnosis

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Background/Aims: I investigated the chromosomal aberrations of patients with pancreatic ductal adenocarcinoma (PDAC) and intraductal papillary mucinous neoplasm (IPMN) by fluorescence in situ hybridization (FISH) analysis to identify the cytogenetic changes and to identify any molecular markers that may useful in the preoperative diagnosis.

Methods: Tissue samples from 48 PDAC and 17 IPMN patients were investigated by FISH analysis using probes directed to chromosome 7q, 17p, 18q, 20q, 21q, and pericentromeric regions to chromosome18 (CEP18).

Results: The PDAC had 17p deletion (95.8%), 18q deletion (83.3%), CEP18 deletion (81.2%), 20q gain (81.2%), 21q deletion (77.1%), and 7q gain (70.8%), and the IPMN had 17p deletion (94.1%), CEP18 deletion (94.1%), 21q deletion (70.6%), 18q deletion (58.8%), 20q gain (58.8%), and 7q gain (58.8%). Significant difference was identified between PDAC and IPMN in the CEP18 gain (p=0.029). Detection of deletion of 17p or 18q gave the highest diagnostic accuracy (80.0%) for PDAC.

Conclusions: The chromosomal alterations were frequently identified in both PDAC and IPMN with similar patterns. The gain of chromosome 18, deletions in 17p and 18q might be involved in the late steps in carcinogenesis of PDAC. The deletion in chromosome 17p and 18q might be an excellent diagnostic markers.

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Keywords: Pancreatic ductal carcinoma; Intraductal papillary mucinous neoplasm;

Fluorescence *in situ* hybridization; Chromosomal aberrations

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer death, and is projected to become the second leading cause of cancer-related death in the United States by 2030.[1] Surgical resection remains the only curative treatment modality; however, most patients are ineligible for surgery because of advanced disease at diagnosis.[2]

To develop effective methods for early stage diagnosis of this deadly disease, which could improve its outcomes, it is important to identify the fundamental genetic changes involved in PDAC carcinogenesis. Activation of the *K-RAS* oncogene and inactivation of the tumor suppressor genes, *CDKN2A/INK4A*, *TP53*, and *SMAD4*, were recently reported to make key contributions to PDAC carcinogenesis.[3] Cytogenetic studies of PDAC have also identified numerous complex structural and numerical alterations at the subchromosomal level, and copy number gain of 3q, 5p, 7p, 8q, 11q, 12p, 17q, 19q, and 20q, and loss of 1p, 3p, 4q, 6q, 8p, 9p, 10q, 12q, 13q, 15q, 17p, 18q, 19p, 21q, and 22q, are recurrent aberrations in PDAC;[4,5] however, cytogenetic studies of PDAC are often complicated by a strong desmoplastic reaction, and inflammatory cells.[6] Accordingly, most analyses have been of pancreatic cell lines, short-term cultures, or xenografted tumor cells; hence recorded genomic alterations could potentially have been acquired *in vitro*.[7] Moreover, most reported chromosomal alterations in PDAC have been from the minority of patients who present without detectable metastases and are eligible for surgery. Also, as a substantial proportion of patients

develop recurrent disease following surgery, detected genetic changes may not represent early events in carcinogenesis.[8] Therefore, studies of PDAC precursor conditions, such as intraductal papillary mucinous neoplasm (IPMN) and pancreatic intraepithelial lesion (PanIN), are essential to identify early events in the process of PDAC carcinogenesis. Recently, an increase in the number of patients diagnosed with IPMN (the most common precursor lesion of PDAC) has been described, likely due to incidental discovery with new imaging techniques; however, only a few studies have reported the genetic alterations in these tumors because of difficulties obtaining tissue samples without using invasive procedures, such as surgery.[9-13]

It is also important for clinicians to discriminate PDAC from other benign pancreatic diseases to facilitate establishment of appropriate therapeutic plans. Endoscopic ultrasound (EUS) guided fine needle aspiration or cytologic brushing is the current standard method for tissue acquisition and pathologic diagnosis of pancreatic lesions; however, histopathologic diagnosis is often difficult and diagnostic sensitivity is consequently unsatisfactory.[14-16] Fluorescence *in situ* hybridization (FISH) is a clinically useful technique, as it can be conducted using limited tissue material, including smears and brushings, and is rapid and relatively accessible.[9] Several studies have reported the clinical utility of FISH analysis using the commercially available probe set, UroVysion (Abbott Molecular Inc, Des Plaines, IL, USA), and the Papanicolaou Society of Cytopathology adopted FISH as a useful ancillary test to complement routine cytology.[17-19] However, The

UroVysion FISH analysis method was developed for diagnosis of bladder cancer using urine samples, and its probe set contains 9p21 which is directed to the *CDKN2A* gene, and chromosome enumeration probes (CEPs) directed to chromosomes 3, 7, and 17. To increase diagnostic accuracy for PDAC, it will be essential to identify the optimal probes targeting chromosomal regions typical of this tumor type.

Most of the previous studies concerning the chromosomal alteration of PDAC and IPMN has been conducted in Western countries, and there has been only a few information about that in Asian countries.[11,12,20,21] In addition, the previous studies did not show consistent chromosomal alteration, which might imply that there could be many genetic variation in the genesis and progression of PDAC and IPMN. We assume that the differences of chromosomal alteration between PDAC and IPMN might provide valuable information about carcinogenesis because IPMN is a well-known premalignant disease; The commonality of genetic alteration between two groups might be presumed to occur in the earlier stages of carcinogenesis, and the differences might be occur in the later steps. Besides, these differences might be also helpful in the preoperative diagnosis of PDAC. In this study, we investigated the chromosomal aberrations in PDAC and IPMN patient samples by FISH analysis using probes targeting chromosomes reported as frequently altered in PDAC, to identify the patterns and differences of chromosomal alteration in Korean PDAC and IPMN patients, and to investigate the optimal probe sets that may aid preoperative diagnosis of PDAC.

Materials and Methods

Patients and sample collection

Prospectively collected tumor tissue samples from patients who underwent pancreatic resection at the Seoul National University Hospital (Seoul, Korea) from April 2015 to July 2016 were investigated. The patients had PDAC (n = 48) or IPMN (n = 17). Among those with IPMN, two, four, and five patients had mild, moderate, and severe dysplasia, respectively, while six had invasive IPMN. Invasive IPMN was defined when there as the presence of an invasive carcinoma derived from (arising in the area of) IPMN pathologically.[22] All 65 patients were scheduled for surgery with a suspicion of malignancy in radiological evaluations. Preoperative histologic diagnoses using EUS guided fine needle aspiration were performed in 25 of 48 (52.1%) PDAC and in 5 of 17 (29.4%) IPMN patients, and were possible to make a diagnosis of malignancy in 19 of 25 (76.0%) PDAC, and in 4 of 5 (80.0%) IPMN patients.

The demographic and pathologic characteristics of the patients, and FISH results for chromosomes 7q, 17p, 18q, 20q, and 21q, and a chromosome enumeration probe 18 (CEP18), were investigated. Pathologic staging was determined according to the seventh edition of the American Joint Committee on Cancer (AJCC) staging system.[23] Informed consent for tissue sample collection for research purposes was obtained from individual patients preoperatively, and the study protocol, as well as ethical issues, were reviewed and approved by the Institutional Review Board at Seoul National University Hospital (IRB No. H-0901-010-267 and H-1807-099-

960). Each tumor sample was harvested immediately after surgical resection and stored in liquid nitrogen. Some of the harvested tumor sample was sent to a pathologist and confirmed by frozen section biopsy to ensure that the tumor tissue was properly harvested.

Seventeen pancreatic cancer cell lines (AsPC-1, Capan-1, Capan-2, MIA PaCa-2, PANC-1, SNU-213, SNU-324, SNU-410, SNU-2466, SNU-2469, SNU-2485, SNU-2491, SNU-2543, SNU-2564, SNU-2570, SNU-2571, and SNU-2608) were obtained from Korean Cell Line Bank (Korean Cell Line Bank, Seoul, Korea). Cells were cultured in RMPI1640, Dulbecco media with 10% heat-inactivated fetal bovine serum at 37°C and 5% CO_2, according to the manufacturer's protocol.

Fluorescence *in situ* hybridization

Tumor samples were transported to the laboratory in an icebox. For FISH examination, tissue samples were minced with a surgical scalpel and incubated in collagenase Type IV (1 mg/mL) (STEMCELL Technologies, Vancouver, BC, Canada) for 20 min. After washing with phosphate-buffered saline (PBS), samples were filtered using 100 μm cell strainers (BD Falcon, Franklin Lakes, NJ, USA) to generate a single cell suspension, followed by centrifugation for 5 min at 1,200 rpm. After adding 5 mL of 0.075 M KCl to each tube, samples were incubated for 25–30 min in a 37°C water bath. Carnoy's fixative (500 μL) was added, and samples were incubated for 5 min at room temperature. Suspensions were centrifuged for 5 min at 1,200 rpm, and supernatants were removed. Pellets were resuspended in 3–5 mL of

Carnoy's fixative and incubated for 20 min at room temperature, then suspensions were centrifuged for 5 min at 1,200 rpm and the supernatants were removed; this step was performed twice. Next, fixed cells were mixed with Carnoy's fixative and dropped onto microscope slides. Air-dried slides were pretreated with 2× standard saline citrate (SSC; 300 mmol/L sodium chloride and 30 mmol/L sodium citrate) for 30 min at 37°C, and dehydrated with cold 70%, 85%, and 100% ethanol for 2 min each. Under protection from light, FISH probes were added to the prepared slides, which were then covered with coverslips and sealed with rubber cement. FISH probes used were as follows: XL Spectrum Orange (7q22)/Spectrum Green (7q36), XL ATM Spectrum Green (11q22)/TP53 Spectrum Orange (17p13), XL MALT Break Apart Spectrum Orange/Green (18q21) (MetaSystems, Altlussheim, Germany), Vysis CEP 18 (D18Z1) Spectrum Orange (Abbott Molecular, Des Plaines, IL, USA), IGH Spectrum Green (14q32.33)/MAFB Spectrum Red (20q12) (Cytocell Ltd, Cambridge, UK), and Vysis RUNX1 Spectrum Green (21q22)/RUNX1T1 Spectrum Orange (8q21) (Abbott Molecular). 17p13 and 18q21 were selected because these probes were directed to *TP53* and *SMAD4*, respectively, which were frequently inactivated tumor suppressor genes in PDAC.[3] Selection of 7q36 and 21q22 probes, which were directed to *DPP6* and *TFF1* gene, respectively, was based on the recent genome-wide association studies from China and Japan.[24,25] 20q12 which was directed to *AIB1* gene was selected because copy number gains were observed in 37% of archival PDAC tissues, and high-level amplification of this gene was reported in four of nine pancreatic cancer cell lines.[26,27] CEP18 was

selected because numerical abnormality of chromosome 18 has been reported as the most common chromosomal alteration in PDAC.[4,6,28,29] Probes and target DNA were simultaneously denatured at 75°C for 5 min, then slides were hybridized for 10 to 16 h at 37°C in a hybridizer (Dako, Glostrup, Denmark). After hybridization, slides were washed in 0.4× SSC at 73°C for 2 min, and in 0.1% Nonidet P-40/2× SSC at room temperature for 2 min. Chromosomes were counterstained with 10 μL of 4′-6′-diamine-2-phenylindole dihydrochloride (DAPI /Antifade) (MetaSystems). Images were analyzed using a Zeiss Axioplan 2 imaging microscope (Carl Zeiss MicroImaging GmbH, Munchen, Germany) with the ISIS software (MetaSystems). Approximately 100 nuclei were scored for each probe (Figure 1). Nuclei with ambiguous signals and cells with poor morphology were excluded from scoring. The absolute cutoff values of FISH analysis using tissue samples has not yet been established, and we adopted the binomial treatment of the data to set the cutoff values because it was one of the reliable methods to calculate the cutoff values of FISH analysis in the hematological diseases. Based on FISH analysis of 18 normal pancreatic tissue samples in a preliminary study, cutoff values for the normal range for FISH analysis were calculated using the Excel 2013 (Microsoft Corporation, Redmond, WA, USA) statistical function CRITBINOM (n, p, α) with a confidence level of 95% (Table 1).[30] For cell lines, the cutoff values of FISH analysis were set as the criteria for bone marrow aspiration in Seoul National University Hospital (Table 2). When the percentage of cells containing > 2 or < 2 FISH signals exceeded the cutoff value, cases were interpreted as positive for polysomy (gain) or

monosomy (deletion), respectively.

Statistical analysis

Categorical variables are presented as numbers and percentages, and were compared using the Fisher's exact test. Continuous variables are expressed as means with standard deviations, and were compared using the Mann-Whitney U test. All statistical analyses were conducted using SPSS 20.0 (SPSS Inc., Chicago, IL, USA), and p-values < 0.05 were considered statistically significant.

Results

Patient demographic and clinicopathological characteristics

The demographic and clinicopathological characteristics of the study population are detailed in Table 2. The mean age of all 65 participants was 65.1 years, and 43 (66.2%) of them were male. Pancreaticoduodenectomy was the most common treatment method, with distal pancreatectomy the second most common. Carbohydrate antigen 19-9 (CA 19-9) levels were significantly higher in the PDAC group than the IPMN group. Pathologically, most patients with PDAC had T3 disease (44/48, 91.7%), and lymph node metastases (37/48, 77.1%). However, there were no statistically differences between the PDAC and invasive IPMN in pTstage (p = 0.080), pN stage (p = 0.173), angiolymphatic invasion (p = 0.413), perineural invasion (p = 0.070), and venous invasion (p = 1.000).

FISH analysis and comparison of PDAC and IPMN

At least two chromosome alterations were detected in all patients with either PDAC or IPMN using standard cutoff values (Table 1). For the PDAC group, 17p deletion was the most frequently detected alteration (46/48, 95.8%), followed by 18q deletion (40/48, 83.3%), CEP18 deletion (39/48, 81.2%), 20q gain (39/48, 81.2%), 21q deletion (37/48, 77.1%), and 7q gain (34/48, 70.8%) (Table 3). For the IPMN group, 17p deletion (16/17, 94.1%) and CEP18 deletion (16/17, 94.1%) were also the most frequently detected alterations, followed by 21q deletion, 20q gain,

18q deletion, and 7q gain. CEP18 gain was significantly more frequent in the PDAC group than the IPMN group (26/48 *vs.* 4/17, $p = 0.029$), and the frequency of 18q deletion was marginally significantly different between the two groups (40/48 *vs.* 10/17, $p = 0.051$). The patterns of chromosomal alteration were similar between invasive and non-invasive IPMN, and there were no statistical differences between the two groups in the chromosomal alterations detected by each probe. The details of frequent chromosomal alterations identified in invasive *vs.* non-invasive IPMN were as follows: 7q gain (4/6 *vs.* 6/11, $p = 1.000$), 17p deletion (6/6 *vs.* 10/11, $p = 1.000$), 18q deletion (3/6 *vs.* 7/11, $p = 0.644$), 20q gain (4/6 *vs.* 7/11, $p = 1.000$), 21q deletion (6/6 *vs.* 6/11, $p = 0.102$), and CEP18 deletion (6/6 *vs.* 10/11, $p = 1.000$).

There were statistically significant differences between the PDAC and IPMN groups in the proportion of cells with 17p deletion (32.2 ± 29.3 *vs.* 16.9 ± 20.8, $p = 0.019$), and 18q deletion (27.7 ± 30.2 *vs.* 7.0 ± 13.5, $p = 0.004$) (Figure 2). For the other probes, there were no statistically significant differences in the chromosomal alteration rates between the PDAC group and IPMN group.

Modification of the cutoff value (percentage of cells positive for a chromosome alteration required for a positive score) resulted in statistically significant differences in mean alteration rates between the PDAC and IPMN groups for some probes, as follows: 17p deletion with cutoff values of 10.0% (36/48 *vs.* 7/17, $p = 0.011$) and 20.0% (27/48 *vs.* 3/17, $p = 0.006$); 18q deletion with cutoff values of 10.0% (29/48 *vs.* 2/17, $p = 0.001$) and 20.0% (23/48 *vs.* 1/17, $p = 0.002$); and CEP18 gain with a cutoff value of 5.0% (18/48 *vs.* 2/17, $p = 0.048$).

Associations of clinicopathologic features and results of FISH analysis

Analysis of relationships between the commonly identified chromosomal alterations and clinicopathological factors demonstrated that CEP18 gain was significantly more frequent in older patients (21/28 *vs.* 7/20, $p = 0.024$) and those with lymph node metastasis (23/37 *vs.* 3/11, $p = 0.041$) (Table 4); however, no other probes exhibited any significant associations with clinicopathological factors.

For patients with PDAC, overall 1 and 2 year survival rates were 67.2% and 61.6%, respectively. During follow-up (median, 15.5 months; range, 0–26 months), recurrence was diagnosed in 26 of 48 (54.2%) patients. The majority of recurrence was diagnosed within 12 months (21/26, 80.8%), and the median recurrence time was 5.0 months. Patients with recurrence had comparable carcinoembryonic antigen (CEA) and CA 19-9 levels and pathologic findings, including T stage, N stage, differentiation, angiolymphatic invasion, perineural invasion, and venous invasion, to those without recurrence (Table 5). There were also no significant differences between patients with recurrence and without recurrence in 7q gain, 17p deletion, 18q deletion, CEP18 deletion, 20q gain, 21q deletion, or CEP18 gain.

Preoperative diagnosis of PDAC

To clarify whether the investigated chromosomal alterations were useful for discriminating between PDAC and IPMN, we evaluated the diagnostic accuracies of various patterns of chromosomal alterations (Table 6). Analysis including the five

common chromosomal aberrations in the PDAC group (7q gain, 17p deletion, 18q deletion, 20q gain, and 21q deletion) and CEP18 gain, which were significantly more frequent than in the IPMN group, identified a mean of 4.6 ± 0.9 (range, 2–6) chromosomal alterations in the PDAC group, with 3.7 ± 1.1 (range, 2–6) in the IPMN group (p = 0.004). Selection of single probes resulted in higher diagnostic accuracy, with values of 79.3% (sensitivity, 87.8%; specificity, 58.8%; and relative risk, 2.5) for 17p deletion (cutoff value, 10.0%), and it slightly increased to 80.0% when 17p deletion (cutoff value, 10.0%) combined with 18q deletion (standard cutoff value) (sensitivity, 97.9%; specificity, 29.4%; and relative risk, 4.8) or 18q deletion with a 10.0% cutoff value (sensitivity, 89.6%; specificity, 52.9%; and relative risk, 2.3).

When we analyzed the 25 PDAC patients who were performed preoperative histologic diagnosis, all of the 6 patients (100.0%) who had not diagnosis of malignancy showed positive results by FISH analysis whether the diagnostic criteria was set to the 17p deletion (cutoff value, 10.0%) combined with 18q deletion (standard cutoff value) , or 18q deletion (cutoff value, 10.0%). The 18 (94.7%), and 16 (84.2%) of 19 patients who had preoperative diagnosis of malignancy showed positive results when the diagnostic criteria was set to the 17p deletion (cutoff value, 10.0%) combined with 18q deletion (standard cutoff value), or 18q deletion (cutoff value, 10.0%), respectively.

Results of FISH analysis for pancreatic cancer cell lines

Chromosomal alterations were also very frequently identified in all pancreatic cancer cell lines (Table 8). 21q deletion (17/17, 100%) and CEP18 deletion (17/17, 100%) were the most frequently detected alterations, followed by 17p deletion (16/17, 94.1%), 18q deletion (16/17, 94.1%), 7q deletion (15/17, 88.2%), and 20q deletion (13/17, 76.4%).

There were statistically significant differences between the cell line and PDAC groups in the proportion of cells with 7q deletion (54.4 ± 39.0 *vs.* 1.0 ± 1.6, $p < 0.001$), 17p deletion (75.3 ± 31.3 *vs.* 32.2 ± 29.3, $p < 0.001$), 18q deletion (87.6 ± 24.7 *vs.* 27.7 ± 30.2, $p < 0.001$), 20q deletion (37.5 ± 33.7 *vs.* 4.4 ± 9.4, $p < 0.001$), 21q deletion (73.7 ± 26.7 *vs.* 10.2 ± 12.1, $p < 0.001$), and CEP18 deletion (76.9 ± 27.7 *vs.* 14.6 ± 16.6, $p < 0.001$). There were no statistical significant differences between the two groups with 7q gain (20.6 ± 35.6 *vs.* 18.4 ± 26.5, $p = 0.498$), 17p gain (5.1 ± 14.5 *vs.* 1.5 ± 7.0, $p = 0.149$), 18q gain (3.0 ± 4.9 *vs.* 2.6 ± 9.2, $p = 0.164$), 20q gain (27.7 ± 36.1 *vs.* 16.5 ± 21.8, $p = 0.869$), 21q gain (4.4 ± 9.2 *vs.* 7.2 ± 16.3, $p = 0.117$), and CEP18 gain. (7.1 ± 20.1 *vs.* 12.2 ± 20.8, $p = 0.340$).

Discussion

The results of the present study demonstrate that chromosomal alterations are very frequent in tumor samples from patients with both PDAC and IPMN. The chromosomal aberration patterns in IPMN were similar to those in PDAC, there were no significant differences in most probe sets, and comparisons of invasive and non-invasive IPMNs also demonstrated no significant differences between these groups. These results imply that similar early genetic alterations may be implicated in the development of both IPMN and PDAC, although they may be partly attributable to the fact that more than half of patients had severe dysplasia or invasive IPMN. Some previous cytogenetic studies support this assumption. Fujii et al.[11] conducted PCR-based microsatellite analysis of 13 IPMN specimens and found frequent loss of heterozygosity at 6q, 8p, 9p, 17p, and 18q with ratios of 31% to 62%. Fritz et al.[10] investigated 20 IPMN specimens by microarray-based comparative genomic hybridization analysis and reported frequent loss of chromosomes 2, 4q, 5q, 6q, 8p, 10q, 11q, 13q, 15q, 18q, and 22q with ratios of 38.5% to 76.9%, and gains of chromosomes 7 and 19q in half of specimens from invasive IPMN or IPMN with severe dysplasia. Both studies identified chromosomal aberrations also frequently identified in PDAC.[4,5] Nevertheless, the chromosomal changes in IPMN have not been fully elucidated and further studies are warranted, as most previous studies have been based on small numbers of tissue samples.

The present study identified that, using a modified cutoff value of 10%, deletions in 17p13 (*TP53*) and 18q21 (*SMAD4/DPC4*) were significantly more frequent in

PDAC than IPMN. Both the *TP53* and *SMAD4/DPC4* genes are well-known tumor suppressors reportedly inactivated in more than 50% of PDACs.[3] Previous studies of genetic or protein loss of *TP53* and *SMAD4* revealed rising incidence with increasing PanIN grade.[31,32] The present study supports the previously proposed tumor progression model for PDAC, which postulates that genetic changes at these loci may be involved in the late steps of carcinogenesis.[33] However, the difference of chromosomal alteration between the PDAC and IPMN might be due to the difference of pathway between PanIN- and IPMN-derived carcinogenesis. The deletion rates of chromosome 17p and 18q have been reported to range from 80% to 100%, and 56% to 88% in PDAC or PanIN with high grade dysplasia, and from 73% to 100%, and 54% to 100% of IPMN with high grade dysplasia or invasion, respectively.[12,13,21,34] However, some other studies reported that the deletion of 17p and 18q even in PanIN-1 in 87% and 50%, respectively, and the *SMAD4/DPC4* gene was inactivated only 3% of IPMN.[13,35] There have been only a small number of studies concerning the chromosomal alteration of the precursor lesions of PDAC, and future studies are necessary to elucidate how it is involved at any stage of the carcinogenesis.

The present study also determined that CEP18 gain was significantly more frequent in PDAC; however, little is known about the significance of changes in chromosome 18 copy number, particularly gain, in pancreatic carcinogenesis. On the contrary, some previous cytogenetic studies reported consistent frequent loss of chromosome 18 in PDAC, which was also identified in the present study.[4,6,8] Gain

of chromosome 18 has been reported in lymphoproliferative diseases, including acute lymphocytic leukemia, multiple myeloma, and non-Hodgkin's lymphoma; however, its role in carcinogenesis and clinical significance has yet to be elucidated.[36] For pancreatic disease, Miyabe et al.[12] reported that polysomy 18 (CEP18) was significantly more frequent in invasive IPMN, and may be involved in malignant transformation of IPMN, along with polysomy 7 and *P16/TP53* deletion. Further studies are necessary to clarify the significance in carcinogenesis and clinical impact of CEP18.

The present study did not reveal clear associations between chromosomal aberrations and clinicopathologic features, including disease recurrence and patient survival, other than for CEP18 gain, which was more frequent in older patients and those with lymph node metastasis. This may be because this study was performed on small patient populations with relatively short-term follow-up. Moreover, approximately 90% of patients had T3 disease and about 80% had lymph node metastases, which could mask the effects of chromosomal alterations; however, there are some reports of a relationship between chromosomal alterations and clinicopathologic prognostic factors. Gutierrez et al.[37] reported that changes of chromosomes 7, 17q, 18q21, and 20 were significantly more frequent in advanced TNM stage tumors, and that numerical changes of chromosomes 4 and 9q34, together with gains of chromosome 8q24, were associated with reduced overall survival of patients. Stoecklein et al.[38] reported that chromosome 17 ploidy level was negatively associated with disease free survival and overall survival.

Although EUS guided cytology and core needle biopsy have been the primary tools for diagnosis of PDAC, they (particularly cytology) have been discredited because of low diagnostic sensitivity. The diagnostic yields of pancreatic EUS guided fine needle aspiration and core needle biopsy indicate sensitivities for these techniques of 54% to 96%, and 71% to 99%, respectively, and the present study showed similar sensitivity (76.0%).[14,15] The main limitation of cytology is false-negative results in patients with PDAC, which can be attributed to various factors, including difficulties in cytologic interpretation of specimens with inflammatory cells, induced by adjacent chronic pancreatitis or recent instrumentation; paucicellular specimens, which harbor few or no malignant cells; and well-differentiated carcinomas, which are difficult to discern.[39] FISH can be used to analyze limited tissue material, including small biopsies, and samples from brushing or aspiration cytology, and has the ability to detect chromosomal alterations common in malignant tumors. This technique has been increasingly used in research and clinical practice for detection of pancreatobiliary malignancy in cytology specimens.[17-20]

The present study revealed the highest diagnostic accuracy for PDAC (80.0%) of FISH tests positive for 17p deletion or 18q deletion, with a cutoff value of 10.0%, and single probe detection of 17p deletion (cutoff value, 10.0%) had a diagnostic accuracy of 79.3%, with acceptable sensitivity (87.8%) and specificity (58.8%). We also found that these probe sets might be helpful in increasing the preoperative diagnostic accuracy for the patients who were failed to diagnose PDAC with

conventional histologic examinations. Over decades, some studies have reported the clinical utility of UroVysion FISH using pancreatobiliary brushing specimens, revealing sensitivity significantly higher than that of conventional cytology for detection of malignancy.[17-20] UroVysion FISH has a diagnostic sensitivity of 34% to 58%, which is higher than that of routine cytology (8% to 40%); however, approximately half of patients with malignancy remain undiagnosed by FISH.[16-20] However, only a few FISH studies of pancreatobiliary malignancies have used probes other than the UroVysion FISH probe set. Miyabe et al.[12] reported that polysomy 7, polysomy 18, *P16* deletion, and *TP53* deletion were significantly more frequent in invasive IPMN, and that detection of polysomy 7 or *TP53* deletion had potential value as diagnostic markers for invasive IPMN. Fritcher et al.[16] reported that the combination of the FISH probes, 1q21, 7p12, 8q24, and 9p21, identifies cancer cells with 93% sensitivity and 100% specificity, and has significantly higher sensitivity (64.7%) than the UroVysion probes (45.9%) or routine cytology analysis (18.8%). The FISH probe sets used in the present study, which target genes associated with PDAC, showed acceptable diagnostic accuracy, and could be useful as an adjunct to conventional histopathologic examination.

Chromosomal alterations were also very frequently identified in the pancreatic cancer cell lines. Deletion was more frequent than gain, and the proportion of cells with deletion was significant higher than PDAC group in all probes. The patterns of chromosomal alteration were slightly different when compared to that of PDAC, and only one cell line (SNU-2491) showed the same chromosomal alteration pattern

in all probes. There are the possibilities of clonal selection and spontaneous chromosomal alterations which could be accumulated during several passages might result in these differences.[40,41] The genetic alteration of surrounding non-tumor cells such as inflammatory cells or fibroblasts in the PDAC tissue samples might also have contributed to the differences.

This study has some limitations. First, as our sample size was relatively small and FISH analysis was performed with relatively few probe sets, the statistical power may be limited. This may account for the failure to elucidate any correlation between chromosomal alterations and clinicopathologic factors, including disease recurrence and patient survival. Second, this study compared chromosomal alterations of PDAC to those of IPMN. Because we aimed to find out the useful FISH probe sets which could aid preoperative histologic diagnosis, we selected the probes directed to the chromosomes which alterations had been reported relatively frequent in PDAC to increase the diagnostic sensitivity, and set IPMN as a control group to identify the discrimination power of selected probes because IPMN might harbor similar chromosomal alterations to PDAC. However, the chromosomal alterations during the PanIN-derived carcinogenesis would be different from that from IPMN-derived pathway. Because we experimented with cryopreseved tissue samples other than paraffin blocks, we could not harvest more premalignant tissue samples of PanIN and IPMN lesions with low to high grade dysplasia, which might make it possible to elucidate the differences of chromosomal alterations during the PanIN- and IPMN-derived pancreatic carcinogenesis. Nevertheless, we believe the

commonality and the differences of chromosomal alteration between PDAC and IPMN identified in the present study could provide helpful information about the carcinogenesis of PDAC for conducting future studies. Lastly, this study was performed using tissue samples obtained by surgical resection, which harbored sufficient cells for analysis. This could have resulted in overestimation of diagnostic accuracy. Therefore further studies are required to apply our findings in clinical practice using limited cytology specimens or small biopsies, and attempts to identify a more specific FISH probe set devoted to detection of chromosomal alterations typical of PDAC are warranted.

In conclusion, chromosomal alterations were frequently identified in both PDACs and IPMNs. PDACs had 17p deletion, 18q deletion, CEP18 deletion, 20q gain, 21q deletion, and 7q gain in more than 70% of patients, and IPMNs had a similar chromosomal aberration pattern; however, IPMNs had a lower positive rate. Gain of chromosome 18 and deletions in 17p and 18q may be involved in the late steps of PDAC carcinogenesis. Although there were no clear clinicopathological associations with chromosomal alterations, deletions at chromosome 17p and 18q may represent excellent diagnostic markers for PDAC.

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Table 1. Cutoff values for each FISH probe.

Probe	Cutoff value (%)		
	Monosomy	Polysomy*	Aneuploidy†
7q22/7q36	1.0	0.0	1.0
17p13	1.0	0.0	1.0
18q21	2.0	1.0	3.0
20q12	1.0	0.0	1.0
21q22	2.0	1.0	3.0
CEP18	3.0	1.0	4.0

*Trisomy cutoff + tetrasomy cutoff. †Monosomy cutoff + polysomy cutoff. CEP18, chromosome enumeration probe 18.

Table 2. Cutoff values for each FISH probe of pancreatic cancer cell lines.

Probe	Cutoff value (%)	
	Monosomy	Polysomy*
7q22/7q36	3.0	0.0
17p13	2.0	3.0
18q21	1.0	1.0
20q12	1.0	0.0
21q22	3.0	4.0
CEP18	-	-

*Trisomy cutoff + tetrasomy cutoff. CEP18, chromosome enumeration probe 18.

Table 3. Patient demographic and clinicopathologic data.

Characteristic	All (n = 65)	PDAC (n = 48)	IPMN (n = 17)	*p*-value
Age (years)	65.1 ± 9.7	65.9 ± 8.6	63.1 ± 12.5	0.617
Sex (male, %)	43 (66.2)	31 (64.6)	12 (70.6)	0.653
Types of operation				0.136
PD	38 (58.5)	29 (60.4)	9 (52.9)	
Distal pancreatectomy	20 (30.8)	16 (33.3)	4 (23.5)	
Other*	7 (10.8)	3 (6.2)	4 (23.5)	
CEA	6.5 ± 22.3	7.9 ± 25.9	2.8 ± 1.1	0.662
CA 19-9	1055.0 ± 2786.1	1396.0 ± 3151.2	32.1 ± 31.9	< 0.001
pT†				0.080
T1	2 (3.7)	1 (2.1)	1 (16.7)	
T2	2 (3.7)	1 (2.1)	1 (16.7)	
T3	48 (88.9)	44 (91.7)	4 (66.7)	
T4	2 (3.7)	2 (4.2)	0 (0.0)	
pN†				0.173
N0	14 (25.9)	11 (22.9)	3 (50.0)	
N1	40 (74.1)	37 (77.1)	3 (50.0)	
Differentiation†				0.010
Well/Moderate/Poor	7/36/8	7/32/8	0/4/0	
Unknown	3	1	2	

Angiolymphatic invasion (+)[†]	26 (48.1)	22 (45.8)	4 (66.7)	0.413
Perineural invasion (+)[†]	44 (81.5)	41 (85.4)	3 (50.0)	0.070
Venous invasion (+)[†]	31 (57.4)	28 (58.3)	3 (50.0)	1.000

[*]Total pancreatectomy (n = 4), subtotal pancreatectomy (n = 2), central pancreatectomy (n = 1).

[†]The described pathologic data and statistical analysis of patients with IPMN only refer to patients with invasive IPMN (n = 6). PDAC, pancreatic ductal adenocarcinoma; IPMN, intraductal papillary mucinous neoplasm; PD, pancreaticoduodenectomy; CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9.

Table 4. Result of FISH analysis according to pathologic diagnosis.

Chromosomal alteration	PDAC (n = 48)	IPMN (n = 17)	*p*-value
7q22/7q36			
Monosomy	14 (29.2)	3 (17.6)	0.523
Polysomy	34 (70.8)	10 (58.8)	0.363
Aneuploidy	38 (79.2)	11 (64.7)	0.326
17p13			
Monosomy	46 (95.8)	16 (94.1)	1.000
Polysomy	10 (20.8)	3 (17.6)	1.000
Aneuploidy	44 (91.7)	16 (94.1)	1.000
18q21			
Monosomy	40 (83.3)	10 (58.8)	0.051
Polysomy	10 (20.8)	3 (17.6)	1.000
Aneuploidy	39 (81.2)	10 (58.8)	0.100
20q12			
Monosomy	27 (56.2)	10 (58.8)	0.854
Polysomy	39 (81.2)	11 (64.7)	0.191
Aneuploidy	44 (91.7)	15 (88.2)	0.648
21q22			
Monosomy	37 (77.1)	12 (70.6)	0.744
Polysomy	17 (35.4)	8 (47.1)	0.397

Aneuploidy	40 (83.3)	14 (82.4)	1.000
CEP18			
Monosomy	39 (81.2)	16 (94.1)	0.270
Polysomy	26 (54.2)	4 (23.5)	0.029
Aneuploidy	47 (97.9)	16 (94.1)	0.458

Patients with positive results are presented as n (%). PDAC, pancreatic ductal adenocarcinoma; IPMN, intraductal papillary mucinous neoplasm; CEP18, chromosome enumeration probe 18.

Table 5. Relationships between clinicopathological parameters of 48 pancreatic cancer patients and fluorescent i*n situ* hybridization results.

Characteristic	N	17p13 monosomy	*p*	18q21 monosomy	*p*	20q12 polysomy	*p*	21q22 monosomy	*p*	7q36 polysomy	*p*	CEP18 polysomy	*p*
Age													
≤ 65	20	19 (95.0)	1.000	17 (85.0)	1.000	17 (85.0)	0.716	17 (85.0)	0.319	13 (65.0)	0.452	7 (35.0)	0.024
> 65	28	27 (96.4)		23 (82.1)		22 (78.6)		20 (71.4)		21 (75.0)		21 (67.9)	
Sex													
Male	31	29 (93.5)	0.533	26 (83.9)	1.000	24 (77.4)	0.460	24 (77.4)	1.000	20 (64.5)	0.320	18 (58.1)	0.464
Female	17	17 (100.0)		14 (82.4)		15 (88.2)		13 (76.5)		14 (82.4)		8 (47.1)	
CEA													
≤ 5.0	37	35 (94.6)	1.000	30 (81.1)	0.667	32 (86.5)	0.081	27 (73.0)	0.091	27 (73.0)	0.456	19 (51.4)	0.475
> 5.0	10	10 (100.0)		9 (90.0)		6 (60.0)		10 (100.0)		6 (60.0)		7 (70.0)	
CA 19-9													

≤ 37.0	10	10 (100.0)	1.000	8 (80.0)	0.666	7 (70.0)	0.370	7 (70.0)	0.675	8 (80.0)	0.701	5 (50.0)	1.000
> 37.0	38	36 (94.7)		32 (84.2)		32 (84.2)		30 (78.9)		26 (68.4)		21 (55.3)	
pT													
T1/T2	2	2 (100.0)	1.000	2 (100.0)	1.000	2 (100.0)	1.000	1 (50.0)	0.410	2 (100.0)	1.000	1 (50.0)	1.000
T3/T4	46	44 (95.7)		38 (82.6)		37 (80.4)		36 (78.3)		32 (69.6)		25 (54.3)	
pN													
N0	11	11 (100.0)	1.000	9 (81.8)	1.000	9 (81.8)	1.000	7 (63.6)	0.246	8 (72.7)	1.000	3 (27.3)	0.041
N1	37	35 (94.6)		31 (83.8)		30 (81.1)		30 (81.1)		26 (70.3)		23 (62.2)	
Differentiation													
Well	7	6 (85.7)	0.518	7 (100.0)	0.552	6 (85.7)	0.866	5 (71.4)	0.281	5 (71.4)	0.592	4 (57.1)	0.816
Mod.	32	31 (96.9)		26 (81.2)		25 (78.1)		26 (81.2)		21 (65.6)		17 (53.1)	
Poor	8	8 (100.0)		6 (75.0)		7 (87.5)		6 (75.0)		7 (87.5)		4 (50.0)	
ALI													
(-)	26	26 (100.0)	0.205	22 (84.6)	1.000	21 (80.8)	1.000	19 (73.1)	0.473	20 (76.9)	0.313	15 (57.7)	0.594
(+)	22	20 (90.9)		18 (81.8)		18 (81.8)		18 (81.8)		14 (63.6)		11 (50.0)	

PNI													
(-)	7	6 (85.7)	0.273	7 (100.0)	0.583	5 (71.4)	0.601	5 (71.4)	0.653	4 (57.1)	0.400	2 (28.6)	0.223
(+)	41	40 (97.6)		33 (80.5)		34 (82.9)		32 (78.0)		30 (73.2)		24 (58.5)	
Venous invasion													
(-)	20	20 (100.0)	0.504	18 (90.0)	0.440	16 (80.0)	1.000	14 (70.0)	0.488	14 (70.0)	0.915	10 (50.0)	0.624
(+)	28	26 (92.9)		22 (78.6)		23 (82.1)		23 (82.1)		20 (71.4)		16 (54.2)	

CEP18, chromosome enumeration probe 18; CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9; ALI, angiolymphatic invasion; PNI, perineural invasion.

Table 6. Clinicopathological differences and fluorescent i*n situ* hybridization test results according to cancer recurrence.

	All (n = 48)	No recurrence (n = 22)	Recurrence (n = 26)	*p*-value
Age (years)	65.9 ± 8.6	66.4 ± 6.3	65.4 ± 10.2	0.868
Sex (male, %)	31 (64.6)	15 (68.2)	16 (61.5)	0.632
Type of operation				0.867
PD	29 (60.4)	14 (63.6)	15 (57.7)	
Distal pancreatectomy	16 (33.3)	7 (31.8)	9 (34.6)	
Other*	3 (6.3)	1 (4.5)	2 (7.7)	
CEA	7.9 ± 25.9	12.4 ± 38.3	4.2 ± 5.5	0.424
CA 19-9	1396.0 ± 3151.2	1682.5 ± 3577.5	1153.6 ± 2790.0	0.521
pT				0.246
T1	1 (2.1)	1 (4.5)	0 (0.0)	
T2	1 (2.1)	1(4.5)	0 (0.0)	
T3	44 (91.7)	19 (86.4)	25 (96.2)	
T4	2 (4.2)	1 (4.5)	1 (3.8)	
pN				0.977
N0	11 (22.9)	5 (22.7)	6 (23.1)	
N1	37 (77.1)	17 (77.3)	20 (76.9)	
Differentiation				0.695
Well/Moderate/Poor	7/32/8	4/15/3	3/17/5	
Unknown	1	0	1	
ALI (+)	22 (45.8)	9 (40.9)	13 (50.0)	0.529
PNI (+)	41 (81.5)	19 (86.4)	22 (84.6)	1.000
Venous invasion (+)	28 (58.3)	12 (54.5)	16 (61.5)	0.624
FISH analysis				
17p13 monosomy (+)	46 (95.8)	22 (100.0)	24 (92.3)	0.493
18q21 monosomy (+)	40 (83.3)	21 (95.5)	19 (73.1)	0.055
CEP18 monosomy (+)	39 (81.2)	18 (81.8)	21 (80.8)	1.000

20q12 polysomy (+)	39 (81.2)	18 (81.8)	21 (80.8)	1.000
21q22 monosomy (+)	37 (77.1)	17 (77.3)	20 (76.9)	0.977
7q36 polysomy (+)	34 (70.8)	15 (68.2)	19 (73.1)	0.710
CEP18 polysomy (+)	26 (54.2)	13 (59.1)	13 (50.0)	0.529

*Total pancreatectomy (n = 1), subtotal pancreatectomy (n = 2). PD, pancreaticoduodenectomy; FISH, fluorescent *in situ* hybridization; CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9; ALI, angiolymphatic invasion; PNI, perineural invasion; CEP18, chromosome enumeration probe 18.

Table 7. Parameters for diagnosis of pancreatic ductal adenocarcinoma using fluorescent i*n situ* hybridization.

Diagnostic criteria	Sensitivity	Specificity	Accuracy	Relative risk
All 6 probes (+)	15.2	100.0	38.1	1.4
≥ 5 probes (+)	60.4	70.6	63.1	1.4
≥ 4 probes (+)	89.6	41.2	76.9	1.9
≥ 3 probes (+)	97.9	17.6	76.9	3.1
17p13 (+)	95.8	5.9	72.3	1.1
18q21 (+)	83.3	41.2	72.3	1.5
7q36 (+)	70.8	41.2	63.1	1.2
CEP18 (+)	54.2	76.5	60.0	1.4
20q12 (+)	81.3	35.3	69.2	1.3
21q22 (+)	77.1	29.4	64.6	1.1
17p13 (10%)* (+)	87.8	58.8	79.3	2.5
17p13 (20%)† (+)	56.3	82.4	63.1	1.5
18q21 (10%)* (+)	60.4	88.2	67.7	1.7
18q21 (20%)† (+)	47.9	94.1	60.0	1.6
17p13 and 18q21 (+)	79.2	47.1	70.8	1.5
17p13 (10%)* and 18q21 (+)	60.4	70.6	63.1	1.4
17p13 (10%)* or 18q21 (+)	97.9	29.4	80.0	4.8
17p13 (20%)† and 18q21 (+)	45.8	82.4	55.4	1.4
17p13 and 18q21 (10%)* (+)	56.3	94.1	66.2	1.7

17p13 and 18q21 (20%)[†] (+)	45.8	94.1	58.5	1.5
17p13 (10%)[*] and 18q21 (10%)[*] (+)	47.9	94.1	60.0	1.6
17p13 (10%)[*] or 18q21 (10%)[*] (+)	89.6	52.9	80.0	2.3
17p13 (10%)[*] or 18q21 (20%)[†] (+)	85.4	58.8	78.5	2.1
17p13 (10%)[*] or CEP18 (+)	89.6	35.3	75.4	1.8
17p13, 18q21, and CEP18 (+)	41.7	88.2	53.8	1.4
17p13 (10%)[*], 18q21 (10%)[*], and CEP18 (+)	22.9	100.0	43.1	1.5
17p13 (10%)[*], 18q21 (20%)[†], and CEP18 (+)	16.7	100.0	38.5	1.4
17p13 (20%)[†], 18q21 (10%)[*], and CEP18 (+)	14.6	100.0	36.9	1.4
17p13 (20%)[†], 18q21 (20%)[†], and CEP18 (+)	8.3	100.0	32.3	1.4
17p13 (10%)[*], 18q21 (10%)[*], or CEP18 (+)	93.8	29.4	76.9	2.1

[*]Upper cutoff value, 10%. [†]Upper cutoff value, 20%. 7q36, 7q36 polysomy; 17p13, 17p13 monosomy; 18q21, 18q21 polysomy; CEP18, CEP18 polysomy; 20q12, 20q12 polysomy; 21q22, 21q22 monosomy; CEP18, chromosome 18 enumeration probe.

Table 8. Result of FISH analysis for pancreatic cancer cell lines.

Cell lines	7q36		17p13		18q21		20q12		21q22		CEP18	
	deletion (%)	gain (%)	deletion (%)	gain (%)	deletion (%)	gain (%)	deletion (%)	gain (%)	deletion (%)	gain (%)	deletion (%)	gain (%)
AsPC-1	87.0	0.0	90.0	0.0	100.0	0.0	45.0	0.0	96.0	0.0	98.0	0.0
Capan-1	100.0	0.0	97.0	0.0	100.0	0.0	95.0	0.0	98.0	0.0	97.0	0.0
Capan-2	52.0	0.0	98.0	0.0	99.0	0.0	23.0	0.0	100.0	0.0	98.0	0.0
MIA PaCa-2	92.0	0.0	76.0	4.0	88.0	8.0	87.0	0.0	47.0	0.0	96.0	4.0
PANC-1	61.0	12.0	80.0	0.0	80.0	4.0	77.0	4.0	91.0	0.0	80.0	6.0
SNU-213	84.0	0.0	94.0	0.0	72.5	12.5	45.0	0.0	80.0	0.0	93.0	0.0
SNU-324	0.0	100.0	0.0	60.0	n/s	n/s	29.0	14.0	47.0	0.0	84.0	0.0
SNU-410	5.0	78.0	79.0	0.0	100.0	0.0	58.0	5.0	72.0	22.0	96.0	0.0
SNU-2466	56.0	0.0	97.0	0.0	97.0	0.0	94.0	2.0	94.0	1.0	48.0	4.0
SNU-2469	98.0	2.0	80.0	10.0	100.0	0.0	10.8	70.0	40.0	0.0	50.0	2.0
SNU-2485	8.0	4.0	100.0	0.0	96.0	1.0	32.5	38.0	9.0	0.0	32.0	0.0
SNU-2491	10.0	30.0	98.0	2.0	90.0	9.0	1.0	68.5	80.0	17.0	98.0	2.0
SNU-2543	96.0	0.0	12.5	7.5	98.0	2.0	20.0	6.0	94.0	4.0	98.0	0.0
SNU-2564	45.0	20.0	42.0	3.0	70.0	15.0	1.0	79.0	63.0	30.0	69.0	9.0
SNU-2570	100.0	0.0	97.5	0.0	100.0	0.0	0.0	80.0	96.0	0.0	94.0	0.0
SNU-2571	30.0	5.0	94.0	0.0	100.0	0.0	20.0	5.0	50.0	0.0	70.0	9.0
SNU-2608	0.0	100.0	45.0	0.0	99.0	0.0	0.0	100.0	96.0	0.0	6.0	84.0

CEP18, chromosome enumeration probe 18.

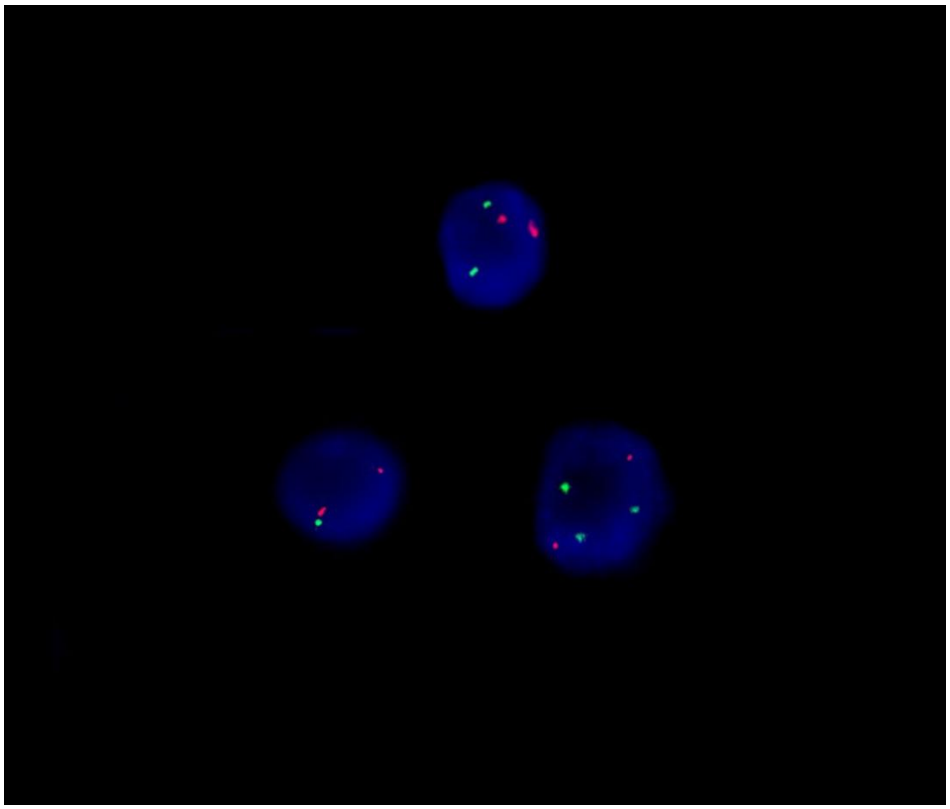

Figure 1. Fluorescent *in situ* hybridization analysis of 21q22 using a colored probe. A normal (disomic) cell is shown with two green signals (top). Heterozygous deletion of 21q22 is shown in the cell with one green signal (bottom left), and heterozygous gain of 21q22 is shown by three green signals (bottom right).

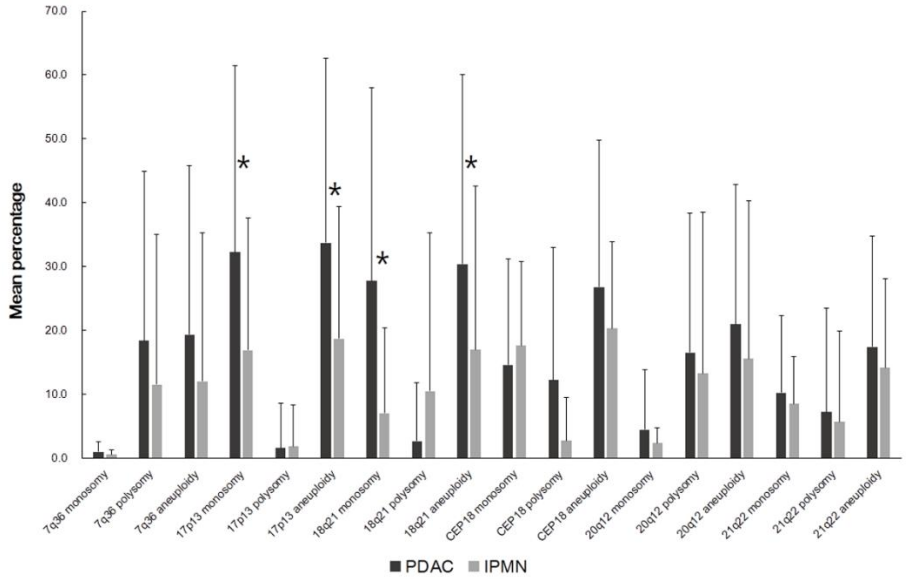

Figure 2. Result of FISH analysis according to the pathologic diagnosis. Genetic alterations in PDAC and IPMN are presented as means and standard deviations. The rate of genetic alteration was higher in the PDAC group than the IPMN group for almost all probes. The difference was significant for 17p13 monosomy (32.2 ± 29.3 *vs.* 16.9 ± 20.8, $p = 0.019$), 17p13 aneuploidy (33.7 ± 28.9 *vs.* 18.7 ± 20.7, $p = 0.030$), 18q21 monosomy (27.7 ± 30.2 *vs.* 7.0 ± 13.5, $p = 0.004$), and 18q21 aneuploidy (30.4 ± 29.7 *vs.* 17.0 ± 25.6, $p = 0.031$). *$p < 0.05$.

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국문 초록

(배경 및 목적) 예후가 매우 불량한 암 중 하나인 췌장암의 치료 성적을 향상시키기 위해서는 췌장암에서 발생되는 유전적 변화를 파악하고, 진단율을 향상시키기 위한 노력이 필수적이다. 본 연구에서는 형광동소보합법을 이용하여 췌장암과 췌장암 전구병변 중 하나인 관내유두상점액성종양에서의 세포유전학적 변화를 살펴보고, 췌장암의 수술적 진단방법으로서의 유용성을 알아보고자 한다.

(대상 및 방법) 2015년 4월부터 2016년 7월까지 서울대학교병원 외과에서 췌장암 (n=48) 및 관내유두상점액성종양(n=17)으로 치료를 받은 65명의 환자에서 수술적 절제직후 종양 조직 시료를 채취했다. 상기 시료들에 대해 염색체 7q, 17p, 18q, 20q, 21q, 그리고, 18번 염색체 동원체(pericentromeric regions to chromosome18) 부위를 표적으로 하는 탐색자(probe)를 사용해 형광동소보합법을 시행했다. 췌장암 및 관내유두상점액성종양 간의 염색체변이의 차이, 염색체 변이와 임상병리학적 인자 및 예후와의 관련성을 조사했고, 탐색자들의 조합을 이용해 수술 전 진단에서의 유용성을 조사했다.

(결과) 췌장암에서의 염색체 변이는 17p 결실(95.8%), 18q 결실(83.3%), CEP18 결실(81.2%), 20q 획득(81.2%), 21q 결실(77.1%), 7q 획득(70.8%) 순

이었고, 관내유두상점액성종양에서는 17p 결실(94.1%), CEP18 결실(94.1%), 21q 결실(70.6%), 18q 결실(58.8%), 20q 획득(58.8%), 7q 획득(58.8%)순이었다. 염색체 변이의 양성율은 췌장암에서 CEP18 획득(26/48 *vs.* 4/17, p = 0.029)이 유의하게 높게 관찰되었으며, 17p 결실과(32.2 ± 29.3 *vs.* 16.9 ± 20.8, p = 0.019), 18q 결실(27.7 ± 30.2 *vs.* 7.0 ± 13.5, p = 0.004)이 발생된 세포들의 비율도 췌장암에서 유의하게 높았다. 염색체 변이와 임상병리학적 인자들 및 예후와의 관련성은 확인되지 않았다. 17p 결실과 18q 결실을 조합했을 때, 췌장암의 수술 전 진단 정확도(80.0%)가 가장 높게 확인되었다.

(결론) 염색체 변이는 췌장암과 관내유두상점액성종양 모두에서 관찰되었으며, 그 양상은 유사하게 나타났다. 18번 염색체의 획득 및 17p, 18q 결실은 췌장암의 발암과정 중 후반기에 발생할 것으로 사료되며, 17p, 18q 결실은 췌장암의 수술 전 진단에 유용한 표지자로 이용될 수 있을 것이다.

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주요어 : 췌장암, 관내유두상점액성종양, 형광동소보합법, 염색체변이

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