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ORIGINAL ARTICLE

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Clonality and K-*ras* mutation analyses of epithelia in intraductal papillary mucinous tumor and mucinous cystic tumor of the pancreas

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Abstract Histological criteria for subclassification of intraductal papillary mucinous tumor (IPMT) and mucinous cystic tumor (MCT) of the pancreas remain ambiguous in the absence of apparent invasion or metastasis. To elucidate this issue, we evaluated clonality and K-*ras* mutations in 11 cystic tumors of the pancreas from female patients, including 7 IPMTs and 4 MCTs. The analyses were performed on DNA from laser microdissected epithelia showing different degrees of atypia as well as normal-appearing epithelia (NAE) in the individual tumors. The grades of atypia were classified into three groups on conventional hematoxylin-eosin staining. Clonality was assessed using the methylation-induced polymorphic inactivation of the X-linked phosphoglycerate kinase gene. The incidence of monoclonality increased with the grades of atypia: 27% for NAE, 43% for grade 1, and 100% for grades 2 and 3. In three of four MCTs, foci of NAE were polyclonal, while monoclonality was seen in each one of grades 1 and 2. The frequency of K-*ras* mutation depended on the grades of atypia: 0% for NAE, 29% for grade 1, 50% for grade 2, and 75% for grade 3. Polyclonal epithelia were devoid of K-*ras* mutation in 92% of sites, while monoclonality was associated with both wild and mutational types in an approximately equal ratio. Both IPMT and MCT seem to arise from polyclonal epithelia and to be replaced by monoclonal neoplastic cells as they undergo dysplastic changes and K-*ras* mutation. These data suggest that the monoclonal expansion precedes K-*ras* mutation.

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Introduction

Cystic tumors of the pancreas have increasingly been detected due to advances in diagnostic imaging technology [11, 14, 32]. Intraductal papillary mucinous tumor (IPMT) and mucinous cystic tumor (MCT) are among the representative cystic neoplasms of the pancreas. IPMT and MCT can be distinguished by their clinicopathological features [13, 23, 35, 45], and invasive characteristics of both tumors seem to be defined or predicted by clinical aspects [13, 42]. However, histopathological criteria for subclassification of IPMT and MCT without invasion remain ambiguous and seem to differ by institution or pathologist. In other words, it may sometimes be difficult to differentiate adenoma from carcinoma in situ, dysplasia from neoplasm, or hyperplasia from dysplasia. IPMT easily extends through the pancreatic duct. This is often a problem when dysplastic epithelia are seen at the surgical margin. In addition, the discrepancy between histological atypia and biological behavior has been pointed out in MCT [6, 31, 40].

To date, there have been few reports studying genetic abnormalities that occur in pancreatic cystic tumors. A stepwise increase in K-*ras* mutation at codon 12 (GGT to GTT or GAT), which is highly associated with pancreatic duct cell carcinoma, has also been found in both IPMT and MCT with increased epithelial dysplasia [2, 18, 30, 33, 39, 44], although several other studies have shown that K-*ras* mutation occurs even in a remarkably high percentage of non-neoplastic tissue [3, 22, 37]. Immunohistochemical overexpression of oncogenes and tumor suppressor genes, such as p53, c-erbB-2, and Dpc-4, has been reported in IPMT [16, 19, 33]. Allelic losses of 3p, 6q, 8p, 9p, 17p and 18q in atypical epithelia in each stage of neoplastic evolution to adenocarcinoma are pointed out in IPMT [10]. However, these somatic alterations do not seem to be essential to the development of IPMT and lack of these alterations in epithelia does not necessarily mean the mass is non-neoplastic.

Neoplasm develops by autonomous growth and is supposed to be monoclonal. Therefore, investigation of clonality is expected to differentiate monoclonal neoplasm from polyclonal non-neoplastic lesions. To date, clonality analysis based on X chromosome inactivation has demonstrated that various neoplastic conditions are monoclonal, and can be used to distinguish neoplasm from non-neoplasm [5, 9, 17, 20, 24, 26, 27, 28].

To elucidate the neoplastic nature of pancreatic cystic tumors we analyzed clonality in epithelia with varying degrees of atypia which constituted IPMT and MCT of female patients. We also examined a correlation between clonality and K-*ras* mutations.

Materials and methods

Definition of IPMT and MCT

The histopathological classification was based on the Atlas of Tumor Pathology, Tumors of the Pancreas [35], in which classifi-

Table 1 Clinicophathological features of intraductal mucinous papillary tumor (IPMT) and mucinous cystic tumor (MCT). *borderline* tumor with moderate dysplasia, *Ph* pancreatic head, *Pb* pancreatic body, *Pt* pancreatic tail

No.	Age	Pathological diagnosis	Site	Size (cm)	Subtype	Communication with duct	Ovarian type stroma	Stromal invasion
1	63	IPMT (carcinoma)	Pt	6.0	Branch	+	–	+
2	71	IPMT (carcinoma)	Pb	24	Main	+	–	+
3	65	IPMT (carcinoma)	Ph	3.0	Main	+	–	–
4	55	IPMT (carcinoma)	Ph	9.0	Main	+	–	+
5	78	IPMT (borderline)	Ph	4.0	Branch	+	–	–
6	53	IPMT (borderline)	Pt	2.0	Branch	+	–	–
7	56	IPMT (borderline)	Ph	4.0	Branch	+	–	–
8	47	MCT (adenoma)	Pb	3,5		–	+	–
9	52	MCT (borderline)	Pt	5.0		Unknown	+	–
10	57	MCT (adenoma)	Pb	3,5		–	+	–
11	41	MCT (adenoma)	Pt	6.0		–	+	–

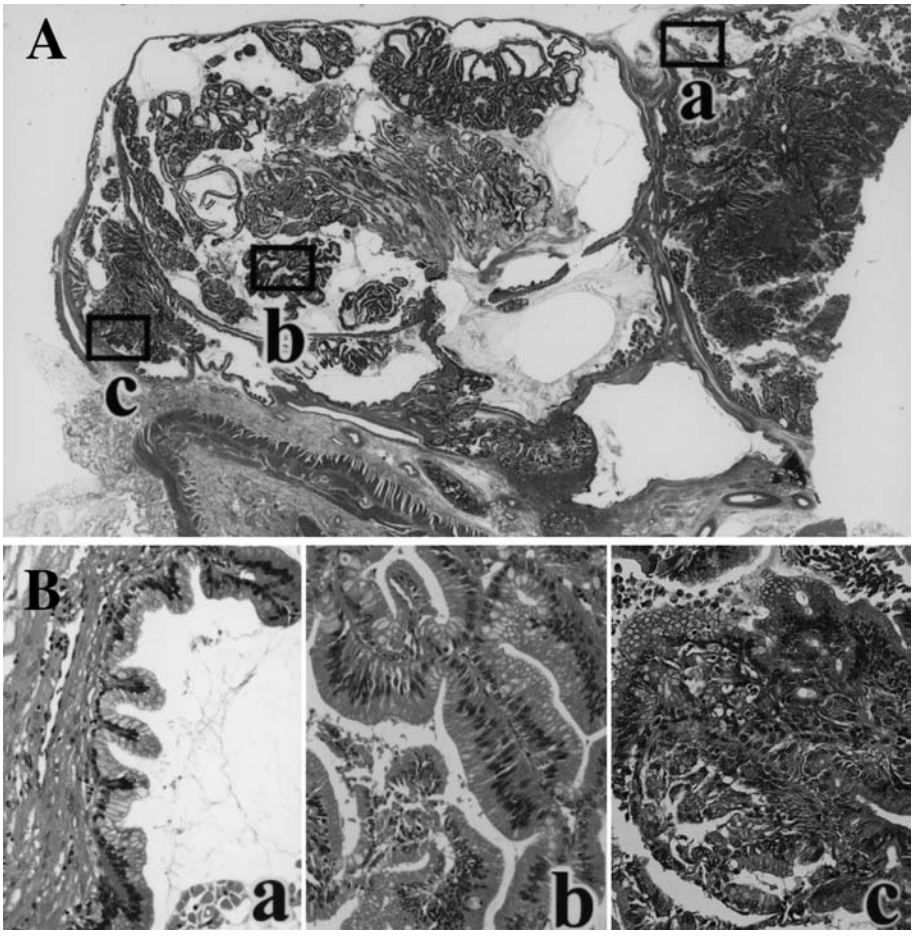

Fig. 1 **A** Low-power microscopic figure of intraductal papillary mucinous tumor (carcinoma) of case 3 in Table 1 and Table 2 (hematoxylin and eosin, ×16). Various grades of epithelial atypia are seen. The letters *a–c* correspond to those in **B**. **B** *a* Grade 1: columnar epithelia and/or papillary structure with nuclei arranged along the basal membrane; *b* grade 2: epithelia with enlarged nuclei and pseudostratification; *c* grade 3: epithelia with a loss of nuclear polarity and cribriforming (hematoxylin and eosin, ×200)

cation is based on World Health Organization (WHO) proposals. Therefore, IPMT was defined as an intraductal pancreatic tumor formed of papillary proliferations of mucin-producing epithelial cells. According to the degree of epithelial dysplasia, IPMT was classified as adenoma, borderline, or carcinoma. MCT was defined as a cystic pancreatic tumor formed of epithelial cells producing mucin, representing gastroenteropancreatic differentiation and an ovarian-type stroma. According to the degree of epithelial dysplasia, MCT was classified as adenoma, borderline, or carcinoma.

Patients

Between 1985 and 2000, we resected cystic pancreatic tumors in 33 patients (23 females and 10 males). The histopathological diagnosis included IPMT for 26 tumors (16 females and 10 males) and MCT for 7 (all females).

Since clonal investigation was based on X chromosome inactivation, as described below, only cases of females were included in the present study. Among the 23 female patients with IPMT or MCT, 11 showed informative polymorphism of the X-linked phosphoglycerate kinase gene (*PGK*; see below). The clinicopathological features of these 11 patients are shown in Table 1.

Histological investigations

We investigated archival materials of resected specimens fixed in 10% formalin and embedded in paraffin. Histological diagnosis was based on 4-μm-thick sections, made from three to six different blocks per patient and stained with hematoxylin and eosin (HE).

Since the lining epithelia of IPMT and MCT demonstrated various degrees of atypia (Fig. 1A), we classified these findings into three grades. Grades 1, 2, and 3 were basically equivalent to PanIN1 (columnar epithelia and/or papillary structure with nuclei arranged along the basal membrane), 2 (epithelia with nuclear crowding, enlarged nuclei, pseudo-stratification and hyperchromatism), and 3 (epithelia with loss of nuclear polarity and true cribriforming, budding of small clusters of epithelial cells into the lumen and luminal necrosis), respectively, as defined by Hruban et al. [15] (Fig. 1B, labeled *a*, *b*, *c*). Lining cells with scanty atypia and flat arrangement were defined as normal-appearing epithelia (NAE) when present within the cystic tumors. Normal epithelia distant from the tumors were sampled as normal control. All the 7 IPMT cases and 1 of the 4 MCT cases demonstrated a diversity of grades in one tumor.

Laser capture microdissection and DNA extraction

The 10-μm-thick samples were cut from formalin-fixed, paraffin-embedded tissues, corresponding to the HE-stained slides for histological investigations described above.

Employing the laser-capture, microdissection system (LCM) (PixCell II, Arcturus, Mountain View, Calif., USA), approximately 200 epithelial cells from each of several serial sections focusing on various grades were harvested to analyze clonality and K-*ras* mutation in the cell population. Dissection with LCM facilitated obtaining only target cells from the specimen with minimal contamination (Fig. 2) [8].

According to the genomic DNA extraction method at the National Institutes of Health (NIH) web site (http://www.nih.gov), the microdissected tissues were digested with proteinase K buffer [0.04% proteinase K, 10 mM Tris-HCl (pH 8.0), 1 mM ethylene diamine tetraacetic acid (EDTA) and 1% Tween-20, final pH 8.0]. The lysate was inactivated for 8 min at 95°C and purified with a DNA purification kit (QIAquick PCR Purification Kit, Qiagen, Valencia, Calif., USA).

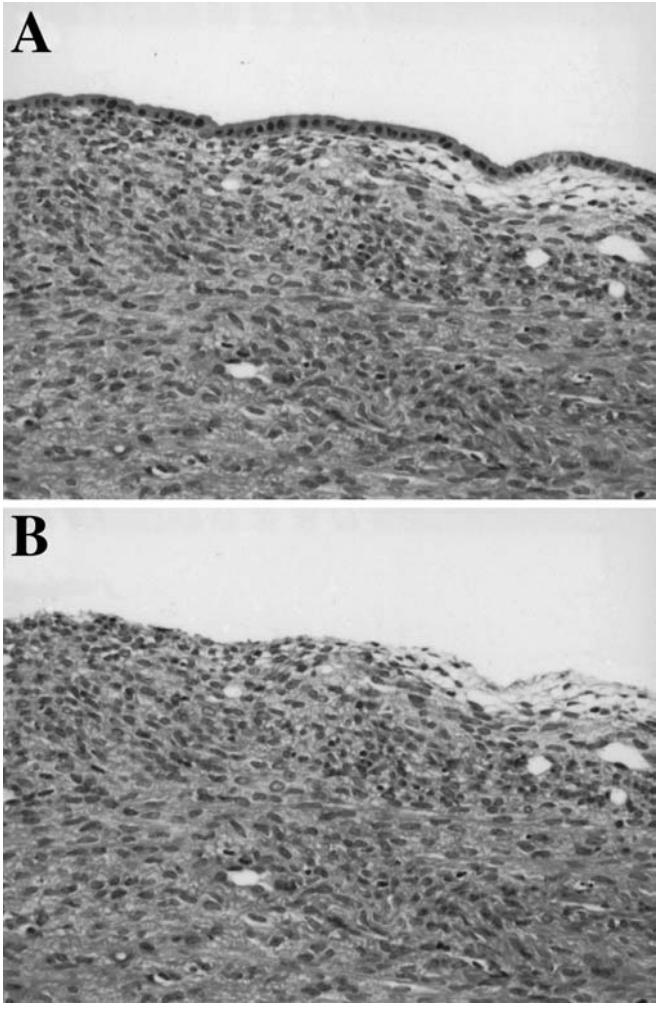

Fig. 2 Laser capture microdissection before (**a**) and after (**b**) microdissection. Foci were harvested with minimal contamination for DNA analysis

Clonality analysis

The purified lysate was used as a template for restriction endonuclease reaction and polymerase chain reaction (PCR). According to the method described by Tamura et al. [38], we evaluated clonality based on methylation-induced inactivation of either allele of X-chromosome-linked *PGK* [4, 7, 21, 34, 41]. Each DNA sample was incubated for 12 h at 37°C in a total volume of 2 μl buffer with 10 U methylation-sensitive restriction endonuclease *Sna*B I [25] (TaKaRa, Tokyo, Japan) to completely digest non-methylated (i.e., activated) alleles. A duplicate sample was subjected to incubation under the same conditions but without *Sna*B I.

The digested and non-digested products were amplified with nested PCR as follows. The first PCR employed 4B (3′-cctgcaaatctctaggcttca-5′) and 3B (3′-gccagcagaacgcctgttac-5′) as primers in 5 μl 10× buffer, 2,5 mM dNTP, 2.5 U *Taq* polymerase (0.5 μl; TaKaRa) for 35 cycles (30 s at 95°C, 30 s at 55°C, 1 min at 72°C) preceded by denaturation for 5 min at 95°C and followed by extension at 72°C. One microliter of the first PCR product was amplified with a second PCR using 4C (3′-cacggaagggaccttgaaa-5′) and 3C (3′-acgcctgttacgtaagctctg-5′) as primers (1.0 pmol each). Second PCR products were precipitated with ethanol. Precipitates were incubated for 12 h at 45°C in a total volume of 20 μl buffer with 10 U of restriction endonuclease *Bst*X I (TaKaRa). The reaction was terminated by heating at 65°C for 20 min, after which the resulting DNA fragments (238 bp, 197 bp) were sub-

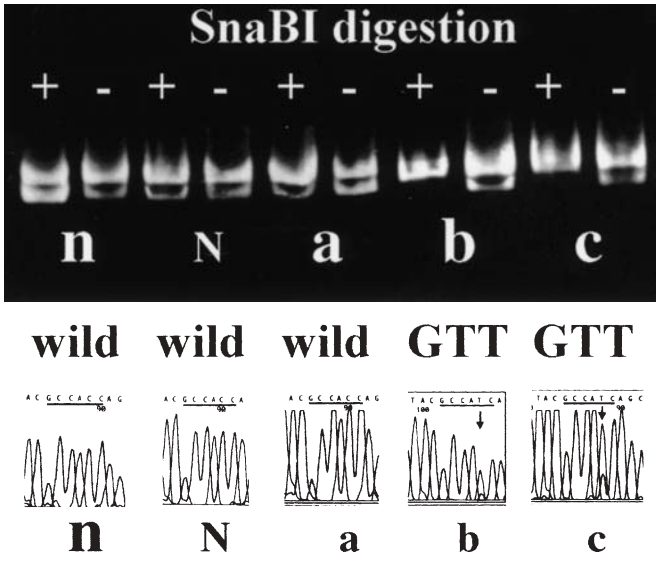

Fig. 3 Clonality assay (*top*) and direct sequencing of K-*ras* at codon 12 (*bottom*) in case 3. *n* Normal duct epithelium distant from intraductal mucinous papillary tumor (IPMT) has polyclonality and K-*ras* (GGT). *N* Normal-appearing epithelia (NAE) within IPMT also has polyclonality and wild K-*ras* (GGT). *a* Grade-1 epithelium within IPMT also has polyclonality and wild K-*ras* (GGT). *b* Grade-2 epithelium exhibits monoclonality and K-*ras* mutation (GTT). *c* Grade-3 epithelium also reveals monoclonality and K-*ras* mutation (GTT)

jected to electrophoresis in an 8% polyacrylamide gel and stained with ethidium bromide (Fig. 3). We confirmed PCR products of *PGK* with an ABI PRISM BigDye Terminator Cycle Sequencing FS Ready Reaction kit (Applied Biosystems, Norwalk, Conn., USA) and an automated sequencer (ABI PRISM 310, Applied Biosystems). We confirmed the reproducibility of clonality twice.

K-*ras* mutation analysis

According to the method described by Fukushima et al. [12], K-*ras* analyses at codons 12 and 13 in all samples were performed with nested PCR as follows. This method has the sensitivity to detect at least one K-ras mutation in 100 cells [12]. The first PCR was carried out with a 50-µl reaction volume containing 1 µl DNA template, 5 µl 10× PCR buffer [100 mM Tris-HCl (pH 8.3), 500 mM KCl, 15 mM $MgCl_2$], 4 µl 2.5 mM dNTP, and primers K1 (3′-taaggcctgctgaaaatg-5′) and K3 (3′-tcaaagaatggtcctgcacc-5′) (1.0 pmol each), and 1.25 U *Taq* polymerase (TaKaRa). The second PCR was performed with primers K5 (3′-actgaatataaacttgtggtagttggagct-5′) and K4 (3′-gatttacctctattgttgga-5′). After the bands (117 bp) were confirmed on an 8% polyacrylamide gel, direct sequencing was performed with an ABI PRISM BigDye Terminator Cycle Sequencing FS Ready Reaction kit (Applied Biosystems) and analyzed on an ABI PRISM 310 Automated sequencer (Applied Biosystems).

Results

Grades of atypia

All seven IPMTs included two to three grades of atypia: three cases contained all grades from 1 to 3 and NAE, while the other four cases showed either grades 1–2 or grades 2–3. Three of four MCTs demonstrated only NAE. The remaining one included grade-1 and grade-2 epithelia and NAE.

Clonality

Even NAE had monoclonality in 27% (3 of 11), and the incidence of monoclonality increased with the grades of atypia: 43% for grade 1 and100% for grades 2 and 3 (Ta-

Table 2 Clonal analysis and K-ras mutations in intraductal papillary mucinous tumor (IPMT) and mucinous cystic tumor (MCT). *borderline* tumor with moderate dysplasia, *NAE* normal-appearing epithelia, *mono* monoclonal, *poly* polyclonal, *wild* wild type (K-ras codon12), / no corresponding focus. There were no K-ras mutations at codon13 in any of the 30 foci in 11cases

No.	Age	Pathological diagnosis		NAE	Grade 1	Grade 2	Grade 3
1	63	IPMT (carcinoma)	Clonality K-ras	Mono Wild	Mono GTT	Mono GTT	Mono GTT
2	71	IPMT (carcinoma)	Clonality K-ras	Poly Wild	Poly Wild	Mono Wild	Mono Wild
3	65	IPMT (carcinoma)	Clonality K-ras	Poly Wild	Poly Wild	Mono GAT	Mono GAT
4	55	IPMT (carcinoma)	Clonality K-ras	Poly Wild	/ /	Mono CGT	Mono CGT
5	78	IPMT (borderline)	Clonality K-ras	Mono Wild	Poly Wild	Mono Wild	/ /
6	53	IPMT (borderline)	Clonality K-ras	Poly Wild	Mono Wild	Mono Wild	/ /
7	56	IPMT (borderline)	Clonality K-ras	Poly Wild	Poly TGT	Mono TGT	/ /
8	47	MCT (adenoma)	Clonality K-ras	Mono Wild	/ /	/ /	/ /
9	52	MCT (borderline)	Clonality K-ras	Poly Wild	mono Wild	Mono Wild	/ /
10	57	MCT (adenoma)	Clonality K-ras	Poly Wild	/ /	/ /	/ /
11	41	MCT (adenoma)	Clonality K-ras	Poly Wild	/ /	/ /	/ /

Table 3 Frequency of monoclonality and K-ras mutations by grade. *IPMT* intraductal papillary mucinous tumor, *MCT* mucinous cystic tumor, *NAE* normal-appearing epithelia

		NAE	Grade 1	Grade 2	Grade 3
IPMT	Monoclonality	2/7 (29%)	2/6 (33%)	7/7 (100%)	4/4 (100%)
	K-ras	0/7 (0%)	2/6 (33%)	4/7 (57%)	3/4 (75%)
MCT	Monoclonality	1/4 (25%)	1/1 (100%)	1/1 (100%)	/
	K-ras	0/4 (0%)	0/1 (0%)	0/1 (0%)	/
Total	Monoclonality	3/11 (27%)	3/7 (43%)	8/8 (100%)	8/8 (100%)
	K-ras	0/11 (0%)	2/7 (29%)	4/8 (50%)	3/4 (75%)

Table 4 Correlation of clonality and K-ras mutation

Clonality	K-ras		
	Wild	Mutation	Total
Polyclonal	11	1	12
Monoclonal	10	8	18
Total	21	9	30

ble 2 and Table 3). In IPMT, five cases that showed polyclonality in NAE acquired monoclonality at atypical grades. Monoclonality at a lower grade was maintained at higher grades, as seen in case 1. This principle of monoclonality progression or maintenance was not applicable in only one case (case 5) where clonality fluctuated from mono- (NAE) to poly- (grade 1) and then to monoclonal state (grade 2).

In MCTs, only one (25%) NAE site showed monoclonality whereas the other three (75%) indicated polyclonality. In case 9, NAE showed polyclonality, and grades 1 and 2 showed monoclonality.

K-*ras* mutation

K-*ras* mutation, if present, was observed at codon 12, but not at codon 13. K-*ras* mutation as well as clonality increased with grades of atypia; 29% for grade 1, 50% for grade 2, and 75% for grade 3, whereas NAE had no K-*ras* mutation (Table 2 and Table 3). The mutation patterns were different in all four IPMTs. The patterns in individual cases remained identical throughout the grade transformation once the mutation appeared. In MCT, there were no cases of K-*ras* mutation even at codon 12.

Correlation of clonality and K-*ras* mutation

The relationships of atypical grades and the incidence of monoclonality and K-*ras* mutation are summarized in Table 3 and Table 4. Compared with K-*ras* mutation, monoclonality was more frequent at each grade of atypia. Polyclonal epithelia were almost invariably devoid of K-*ras* mutation (92% [11 of 12]) while monoclonality was associated with both wild and mutational types in an approximately equal ratio (56% [10 of 18] vs 44% [8 of 18]) (Table 4).

Discussion

A number of investigators have reported that clonal analysis can offer a contributory means of differential diagnosis for tumor-like lesions that are clinically and histologically difficult to diagnose as neoplasm or non-neoplasm [5, 17, 20, 24, 26, 27, 28]. Therefore, we applied this method to pancreatic cystic tumors including IPMT and MCT. Theoretically, clonal analysis can be performed for women either by *PGK* or human androgen receptor gene (*HUMARA*) so long as they are heterozygous or informative with different polymorphisms derived from parents. However, *PGK* was far superior to *HUMARA* in our preliminary experiments in terms of the stability of data obtained from formalin-fixed, paraffin-embedded materials. In our study of *HUMARA*, since several non-specific bands emerge on PCR assay, the judgement of clonality is difficult and unstable. A demerit of *PGK* analysis, relative to *HUMARA*, is a lower percentage of informative cases among the Japanese female population (*PGK* 50% vs *HUMARA* 90%) [27]. In the present series we found that 48% (11 of 23) of female patients with IPMT/MCT were informative.

Our initial hypothesis before the present clonality analysis was that NAE and epithelia of grade 1 in IPMT would be non-neoplastic and all grades of epithelia in MCT were neoplastic. The results were somewhat contrary to our expectation.

All IPMTs and one MCT demonstrated several grades of atypia, and the incidence of monoclonality increased with the grade, reaching 100% for grades 2 and 3. On conventional HE-stained sections, grades 2 and 3 of atypia may be interpreted as adenoma and carcinoma, respectively. Therefore, the conventional diagnosis of adenoma or carcinoma seems to be confirmed by the present clonality analysis.

However, NAE and epithelia of grade 1, conventionally interpreted as “epithelia with no or scanty atypia” and “hyperplastic” epithelia, respectively, showed monoclonality in some cases, suggesting that the monoclonal foci may represent neoplasms whereas polyclonal foci may not be neoplastic. The mixture of monoclonal and polyclonal features in NAE and epithelia of grade 1 seems to indicate tumorigenesis from polyclonal cells with no or scanty atypia accompanied by monoclonal cells progressing to a definite neoplasm of adenoma or carcinoma. This progression theory may be supported by the finding that monoclonality in lower grade epithelia was almost invariably maintained at higher grades (cases 1, 2, 3, 4, 6 and 9).

The above discussion is mainly based on findings of IPMT and can be applied to MCT for the most part. However, MCT deserves some further comment. Three of four MCTs had only NAE, and two of the three showed polyclonality. Despite polyclonality and normal appearance in these two cases, the conventional histological diagnosis was adenoma. This is apparently because the histological diagnosis of MCT was made not by cellular or structural atypism of epithelia, but by the presence of ovarian-like stroma [43]. The traditional concept of MCT has been such that the tumor is definitely neoplastic. Riddler et al. speculated that MCT might be a hamartoma with dispersed sex-cord stroma [29]. Zamboni et al. also hypothesized that MCT might originate from ovarian residues [45]. At any rate, the issue of whether MCT is neoplastic or not, at least at the early stage, seems to require reappraisal. Our results tend to support that histological examination accurately predicts prognosis in MCT [42, 45]. Commencement from polyclonal cells followed by replacement by monoclonal neoplastic cells might occur in MCT, in the same way as suggested for IPMT. However, to confirm the validity of this speculation more data on MCT are needed.

K-*ras* mutation, a key genetic abnormality in pancreatic cancer [1, 36], was also detected in IPMT, but its frequency depended on the grades of atypia. In the present study, there were no cases of MCT showing K-*ras* mutation probably due to the lower grades of atypia and the very small sample size. In this study, as previously reported, mutation patterns in IPMT seem to differ from those in the common type of duct cell carcinoma of the pancreas (mostly GAT, occasionally GTT, and exceptionally other kinds) [2, 18, 30, 33, 39]. It is to be noted that those cases of IPMT that had K-*ras* mutation did not show a change in pattern even when the grade of atypia increased. However, K-*ras* mutation was less frequent than monoclonality at all grades of atypia in IPMT. This means that monoclonality is superior to K-*ras* mutation as a more sensitive marker to detect neoplastic characteristics.

In conclusion, both IPMT and MCT seem to arise from polyclonal epithelia and to be replaced by monoclonal neoplastic cells as they undergo dysplastic changes and K-*ras* mutation. These data show that monoclonal expansion precedes K-*ras* mutation.

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References

1. Almouera C, Shibata D, Forrester K, Martin J, Arheim N, Perucho M (1988) Most human carcinomas of the exocrine pancreas contain mutant c-K-*ras* genes. Cell 53:549–554
2. Bartsch D, Bastian D, Barth P, Schudy A, Nies C, Kisker O, Wagner HJ, Rothmund M (1998) K-ras oncogene mutations indicate malignancy in cystic tumors of the pancreas. Ann Surg 228:79–86
3. Berger DH, Chang H, Wood M, Huang L, Heath CW, Lehman T, Ruggeri BA (1999) Mutational activation of K-*ras* in nonneoplastic exocrine pancreatic lesions in relation to cigarette smoking status. Cancer 85:326–332
4. Busque L, Gilliland DG (1996) The PGK-PCR clonality assay (PPCA). Mol Biotechnol 5:275–280
5. Chung DH, Kang GH, Kim WH, Ro JY (1999) Clonal analysis of a solitary follicular nodule of the thyroid with the polymerase chain reaction method. Mod Pathol 12:265–271
6. Compagno J, Oertel JE (1978) Mucinous cystic neoplasms of the pancreas with overt and latent malignancy (cystadenocarcinoma and cystadenoma). A clinicopathologic study of 41 cases. Am J Clin Pathol 69:573–580
7. Doherty T, Connell J, Stoerker J, Markham N, Shroyer AL, Shroyer KR (1995) Analysis of clonality by polymerase chain reaction for phosphoglycerate kinase-1. Heteroduplex generator. Diagn Mol Pathol 4:182–190
8. Emmert-Buck MR, Bonner RF, Smith PD, Chuaqui RF, Zhuang Z, Goldstein SR, Weiss RA, Liotta LA (1996) Laser capture microdissection. Science 274:998–1001
9. Fearon ER, Hamilton SR, Vogelstein B (1987) Clonal analysis of human colorectal tumors. Science 238:193–197
10. Fujii H, Inagaki M, Kasai S, Miyokawa N, Tokusashi Y, Gabrielson E, Hruban RH (1997) Genetic progression and heterogeneity in intraductal papillary-mucinous neoplasms of the pancreas. Am J Pathol 151:1447–1454
11. Fukukura Y, Fujiyoshi F, Sasaki M, Inoue H, Yonezawa S, Nakajo M (2000) Intraductal papillary mucinous tumors of the pancreas: thin-section helical CT findings. AJR Am J Roentgenol 174:441–447
12. Fukushima N, Suzuki M, Fukayama M (1998) Analysis of K-ras oncogene mutation directly applied to atypical cell clusters on cytologic smear slides of bile and pancreatic juice. Pathol Int 48:33–40
13. Fukushima N, Mukai K, Sakamoto N, Hasebe T, Shimada K, Kosuge T, Kinoshita T, Hirohashi S (2001) Invasive carcinoma derived from intraductal papillary-mucinous carcinoma of the pancreas: clinicopathologic and immunohistochemical study of eight cases. Virchows Arch 439:6–13
14. Gress F, Gottlieb K, Cummings O, Sherman S, Lehman G (2000) Endoscopic ultrasound characteristics of mucinous cystic neoplasms of the pancreas. Am J Gastroenterol 95:961–965
15. Hruban RH, Adsay NV, Albores-Saavedra J, Compton C, Garrett ES, Goodman SN, Kern SE, Klimstra DS, Klöppel G, Longnecker DS, Lüttges J, Offerhaus GJ (2001) Pancreatic intraepithelial neoplasia: a new nomenclature and classification system for pancreatic duct lesions. Am J Surg Pathol 25:579–586
16. Iacobuzio-Donahue CA, Klimsta DS, Adsay NV, Wilentz RE, Argani P, Sohn TA, Yeo CJ, Cameron JL, Kern SE, Hruban RH (2000) Dpc-4 protein is expressed in virtually all human intraductal papillary mucinous neoplasms of the pancreas. Comparison with conventional ductal adenocarcinomas. Am J Pathol 157:755–761
17. Jimbo H, Hitomi Y, Yoshikawa H, Yano T, Momoeda M, Sakamoto A, Tsutsumi O, Taketani Y, Esumi H (1997) Evidence for monoclonal expansion of epithelial cells in ovarian endometrial cysts. Am J Pathol 150:1173–1178
18. Jimenez RE, Warshaw AL, Z'graggen K, Hartwig W, Taylor DZ, Compton CC, Fernandez-del Castillo C (1999) Sequential accumulation of K-*ras* mutations and p53 overexpression in the progression of pancreatic mucinous cystic neoplasms to malignancy. Ann Surg 230:501–509
19. Kawahira H, Kobayashi S, Kaneko K, Asano T, Ochiai T (2000) p53 protein expression in intraductal papillary mucinous tumors (IPMT) of the pancreas as an indicator of tumor malignancy. Hepatogastroenterology 47:973–977
20. Kim H, Piao Z, Park C, Chung WY, Park CS (1998) Clinical significance of clonality in thyroid nodules. Br J Surg 85: 1125–1128
21. Lee ST, McGlennen RC, Litz CE (1994) Clonal determination by the fragile X (FMR1) and phosphoglycerate kinase (PGK) genes in hematological malignancies. Cancer Res 54:5212–5216

22. Lüttges J, Reinecke-Lüthge A, Möllmann B, Menke MA, Clemens A, Klimpfinger M, Sipos B, Klöppel G (1999) Duct changes and K-*ras* mutations in the disease-free pancreas: analysis of type, age relation and spatial distribution. Virchows Arch 435:461–468
23. Lüttges J, Zamboni G, Longnecker D, Klöppel G (2001) The immunohistochemical mucin expression pattern distinguishes different types of intraductal papillary mucinous neoplasms of the pancreas and determines their relationship to mucinous noncystic carcinoma and ductal adenocarcinoma. Am J Surg Pathol 25:942–948
24. Nanba H, Matsuo K, Fagin JA (1990) Clonal composition of benign and malignant human thyroid tumors. J Clin Invest 86: 120–125
25. Nelson M, Raschke E, McClelland M (1993) Effect of site specific methylation on restriction endonuclease and DNA modification methyltransferases. Nucleic Acids Res 21:3139–3154
26. Niho S, Yokose T, Suzuki K, Kodama T, Nishiwaki Y, Mukai K (1999) Monoclonality of atypical adenomatous hyperplasia of the lung. Am J Pathol 154:249–254
27. Noguchi S, Motomura K, Inaji H, Imaoka S, Koyama H (1992) Clonal analysis of human breast cancer by means of the polymerase chain reaction. Cancer Res 52:6594–6597
28. Noguchi S, Motomura K, Inaji H, Imaoka S, Koyama H (1994) Clonal analysis of parathyroid adenomas by means of the polymerase chain reaction. Cancer Lett 78:93–97
29. Riddler GJ, Maschek H, Flemming P, Nashan B, Klempnauer J (1998) Ovarian-like stroma in an invasive mucinous cystadenocarcinoma of the pancreas positive for inhibin. A hint concerning its possible histogenesis. Virchows Arch 432:451–454
30. Sakai Y, Yanagisawa A, Shimada M, Hidaka E, Seki M, Tada Y, Harada T, Saisho H, Kato Y (2000) K-ras gene mutations and loss of heterozygosity at the p53 gene locus relative to histological characteristics of mucin-producing tumors of the pancreas. Hum Pathol 31:795–803
31. Sarr MG, Carpenter HA, Prabhaker LP, Orchard TF, Hughes S, van Heeden JA, DiMagno EP (2000) Clinical and pathologic correlation of 84 mucinous cystic neoplasms of the pancreas: can one reliably differentiate benign from malignant (or premalignant) neoplasms? Ann Surg 231:205–212
32. Scott J, Martin I, Redhead D, and Hammond P, Garden OJ (2000) Mucinous cystic neoplasms of the pancreas: imaging features and diagnostic difficulties. Clin Radiol 55:187–192
33. Sessa F, Solcia E, Capella C, Bonato M, Scarpa A, Zamboni G, Pellegata NS, Ranzani GN, Rickaert F, Klöppel G (1994) Intraductal papillary-mucinous tumours represent a distinct group of pancreatic neoplasms: an investigation of tumour cell differentiation and K-*ras*, p53 and c-erbB-2 abnormalities in 26 patients. Virchows Arch 425:357–367
34. Shroyer KR, Gudlaugsson EG (1994) Analysis of clonality in archival tissues by polymerase chain reaction amplification of PGK-1. Hum Pathol 25:287–292
35. Solcia E, Cappella C, Köppel G (1997) Tumor of the pancreas. (AFIP atlas of tumor pathology. Third series. Fascicle 20) Armed Forces Institute of Pathology Washington D.C.
36. Tada M, Yokosuka O, Omata M, Ohto M, Isono K (1990) Analysis of ras gene mutations in biliary and pancreatic tumors by polymerase chain reaction and direct sequencing. Cancer 66:930–935
37. Tada M, Ohashi M, Shiratori Y, Okudaira T, Komatsu Y, Kawabe T, Yoshida H, Machinami R, Kishi K, Omata M (1996) Analysis of K-*ras* gene mutation in hyperplastic duct cells of the pancreas without pancreatic disease. Gastroenterology 110:227–231
38. Tamura M, Fukaya T, Murakami T, Uehara S, Yajima A (1998) Analysis of clonality in human endometriotic cysts based on evaluation of X chromosome inactivation in archival formalin fixed, paraffin-embedded tissue. Lab Invest 78: 213–218
39. Tateishi K, Tada M, Yamagata M, Isayama H, Komatsu Y, Kawabe T, Shiratori Y, Omata M (1999) High proportion of mutant K-*ras* gene in pancreatic juice of patients with pancreatic cystic lesions. Gut 45:737–740
40. Thompson LD, Becker RC, Przygodzki RM, Adair CF, Heffess CS (1999) Mucinous cystic neoplasm (mucinous cystadenocarcinoma of low-grade malignant potential) of the pancreas: a clinicopathologic study of 130 cases. Am J Surg Pathol 23:1–16
41. van Kamp H, Jansen R, Willemze R, Fibbe WE, Landegent JE (1991) Studies on clonality by PCR analysis of the PGK-1 gene. Nucleic Acids Res 25:2794
42. Wilentz RE, Albores-Saavedra J, Zahurak M, Talamini MA, Yeo CJ, Cameron JL, Hruban RH (1999) Pathologic examination accurately predicts prognosis in mucinous cystic neoplasms of the pancreas. Am J Surg Pathol 23:1320–1627
43. Wilentz RE, Albores-Saavedra J, Hruban RH (2000) Mucinous cystic neoplasms of the pancreas. Semin Diagn Pathol 17: 31–42
44. Yanagisawa A, Kato Y, Ohtake K, Kitagawa T, Ohashi K, Hori M, Takagi K, Sugano H (1991) c-Ki-ras point mutations in ductectatic-type mucinous cystic neoplasms of the pancreas. Jpn J Cancer Res 82:1057–1060
45. Zamboni G, Scarpa A, Bogina G, Iacono C, Bassi C, Talamini G, Sessa F, Capella C, Solcia E, Rickaert F, Mariuzzi GM, Klöppel G (1999) Mucinous cystic tumors of the pancreas: clinicopathological features, prognosis, and relationship to other mucinous cystic tumors. Am J Surg Pathol 23:410–422