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Reappraisal of the Immunophenotype of Pancreatic Intraductal Papillary Mucinous Neoplasms (IPMNs)—Gastric Pyloric and Small Intestinal Immunophenotype Expression in Gastric and Intestinal Type IPMNs—

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Pancreatic intraductal papillary mucinous neoplasms (IPMNs) are mucin-producing neoplasms of the main and/or branch pancreatic ducts. To assess differences between various IPMN subtypes, immunohistochemical markers of gastric surface mucous cells (MUC5AC), gastric gland mucous cells (MUC6 and GlcNAcα1→4Galβ→R), gastric pyloric and duodenal epithelial cells (PDX1), intestinal cells (MUC2 and CDX2), small intestinal cells (CPS1) and large intestinal cells (SATB2) were evaluated in 33 surgically treated IPMNs. MUC2 expression classified IPMNs into gastric (n=17), intestinal (n=8) and mixed gastric and intestinal type (collision=7, composite=1). No differences in age or sex were observed among these types. MUC5AC and PDX1 were expressed in all IPMNs. MUC6 expression was higher in gastric and mixed types than in intestinal type. GlcNAcα1→4Galβ→R was detected in gastric and mixed type, but not in intestinal type. MUC2 and CDX2 expression were higher in intestinal type than gastric and mixed type. CPS1 expression was higher in intestinal type than gastric type. SATB2 was not observed in any IPMNs. Frequent abrupt transition between the two IPMN types in mixed-type IPMNs was observed. Gastric pyloric and small intestinal differentiation are characteristic of gastric and intestinal type IPMN, respectively, and these two IPMN types may have distinct pathogenesis.

Key words: CPS1, CDX2, intraductal papillary-mucinous neoplasm, pancreas, PDX1

I. Introduction

Pancreatic intraductal papillary mucinous neoplasms (IPMNs) are intraductal mucin-producing neoplasms with cystic dilatation of the main and/or branch pancreatic ducts [2, 3, 10, 14, 36, 39]. IPMNs exhibit a spectrum of dysplasia ranging from low to high grade and some IPMNs are also associated with invasive carcinoma. Thus IPMNs are recognized as precursors to pancreatic ductal carcinoma [2, 3, 10, 14, 36, 39]. IPMNs are classified into four subtypes on the basis of morphology, mucin histochemistry [20] and immunohistochemical expression of mucin core protein (MUC) [1, 5, 10, 13, 26, 42]: gastric, intestinal, pancreatobiliary and oncocytic. The two major histological subtypes exhibiting distinctive pathological features are gastric-type and intestinal-type, while pancreatobiliary and oncocytic types represent more rare forms [10, 36, 39]. Gastric-type IPMN is characteristically found in the branch-duct, simulating gastric foveolar epithelium, and exhibit generally low- or intermediate-grade dysplasia [3, 5, 10, 20, 26, 36, 39, 42]. Conversely, intestinal-type IPMN is characteristically found in the main-duct, and typically exhibits intermediate- or high-grade dysplasia, resembling villous tumors of the large intestine [1, 3, 5, 20, 26, 36, 39, 42].

Neoplastic cells in the gastrointestinal tract express several phenotypic markers including mucins, transcription

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factors and enzymes found on normal equivalent cells. Mucins are high-molecular-weight glycoproteins consisting of a protein backbone (MUC), which are named according to their corresponding genes [23], and oligosaccharide chains attached to MUCs. MUCs are expressed in a cell type-specific manner in normal gastrointestinal mucosa. Thus, MUC5AC, MUC6, and MUC2 are expressed in gastric surface mucous cells, gastric gland mucous cells, and intestinal goblet cells, respectively [6, 25]. In addition, N-acetylglucosaminα1→4galactoseβ→R (GlcNAcα1→4Galβ →R) recognized by the HIK1083 monoclonal antibody (Kanto Chemical, Tokyo, Japan) is a sugar residue specific for mucins produced by the gastric gland mucous cells [15, 25, 29, 30].

Homeobox transcription factors control cell growth and differentiation. The development of the gastric antrum in addition to the pancreas and duodenum is regulated by the pancreatic-duodenal homeobox 1 (*PDX1*) gene [11, 37]. In mice and humans, PDX1 is expressed in the nuclei of epithelial cells of the gastric antrum, pancreatic islets, and the duodenum [11, 16, 27, 33, 37]. Intestinal development is regulated by the caudal homeobox 2 (*CDX2*) gene, and CDX2 is expressed in the nuclei of normal intestinal epithelial cells, intestinal metaplastic cells, and neoplastic cells with intestinal differentiation [4, 9]. In the human gastrointestinal tract, the special AT-rich sequence-binding protein 2 (SATB2), a nuclear matrix-associated transcription factor [38], is specifically expressed in colorectal mucosa and in colorectal carcinoma [19].

Monoclonal antibody hepatocyte paraffin 1 (Hep Par 1) is widely used to determine hepatocellular differentiation [40] and the antigen for Hep Par1 was identified as carbamoyl phosphate synthetase 1 (CPS1) [7]. CPS1 is an enzyme in the urea cycle and is located in the mitochondria of normal and neoplastic hepatocytes and small intestinal absorptive cells and goblet cells [8, 18].

In the present study, we examined characteristics of IPMN epithelial cells by immunohistochemical analysis of cell-type specific mucins (MUC5AC, MUC6, MUC2 and GlcNAcα1→4Galβ→R), transcription factor proteins (CDX2, PDX1 and SATB2), and CPS1 to gain a better understanding of the phenotypic differences between gastric- and intestinal-type IPMN and their histogenesis.

II. Materials and Methods

Tissue samples

Thirty-three consecutive cases of IPMN and three cases of normal pancreatic tissue obtained from distal pancreatectomy (for gastric cancer) or pancreatoduodenectomy (for bile duct cancer) were retrieved from pathology files at the Department of Laboratory Medicine, Shinshu University Hospital, Matsumoto, Japan and its affiliated hospital between 1989–2011. Pathologic diagnosis and histological grading of IPMN specimens were performed according to the World Health Organization (WHO) classification of tumors of the digestive system [2]. Hematoxylin and eosin (H&E)-stained archive slides were reviewed and one representative paraffin block containing the tumor area best reflecting the general features of the tumor was selected for immunohistochemical analysis in each case. Pancreatobiliary- and oncocytic-type IPMN were not included in this study. This study was approved by the ethics committee of Shinshu University, Japan.

Histological evaluation

Six histological features were evaluated: (1) predominant localization, (2) the presence of intraluminal nodular growth, (3) pyloric gland-like structures, (4) atrophy of the surrounding pancreas tissue, (5) histological grade, and (6) the occurrence of invasive carcinoma [5]. Based on their predominant localization on histology, IPMNs were classified as either main-duct type or branch-duct type. Briefly, main-duct type predominantly involves the main pancreatic duct with or without extension into branch pancreatic ducts, whereas branch-duct type predominantly involves vice versa. The histological grade of non-invasive neoplasm components were designated into one of two categories; low grade dysplasia (low or intermediate dysplasia on the basis of criteria set by the WHO [2]) and high grade dysplasia (high grade dysplasia on the basis of criteria set by WHO [2]), on the basis of the highest degree of atypia.

Immunohistochemical analysis

All materials were fixed in 10% neutral buffered formalin and embedded in paraffin. Serial tissue sections (3-μm thickness) were prepared from paraffin blocks and stained with H&E for histological examination or used for immunohistochemical staining. To investigate the characteristics of gastrointestinal epithelial cells in IPMNs and normal pancreatic tissue, the following cell lineage-specific markers were used: anti-MUC5AC (Novocastra/Leica Biosystems, Kista, Sweden) for gastric surface mucous cells, anti-MUC6 (Novocastra/Leica Biosystems) and anti-GlcNAcα1→4Galβ→R (clone HIK1083, Kanto Chemical, Tokyo, Japan) for gastric gland mucous cells, anti-PDX1 (guinea pig anti-mouse Pdx1, generously provided by Christopher V.E. Wright, DPhil, Vanderbilt University Medical School, Nashville, TN) [12] for gastric pyloric and duodenal epithelial cells, anti-MUC2 (Novocastra/Leica Biosystems) for intestinal goblet cells, anti-CDX2 (BioGenex Inc. San Ramon, CA) for intestinal epithelial cells, anti-CPS1 (Dako, Carpentaria, CA) for small intestinal absorptive cells and goblet cells, and anti-SATB2 (Santa Cruz Biotechnology, Santa Cruz, CA) for colorectal epithelial cells. Incubation with primary antibodies was performed overnight at 4°C. Immunohistochemical staining was performed using the immunoenzyme polymer method (Histofine Simple Stain MAX PO Multi, Nichirei Biosciences, Tokyo, Japan) with 3,3'-diaminobenzidine as the chromogen. For PDX1 immunostaining, rabbit anti-guinea pig immunoglobulin (Dako) was used as the secondary

Table 1. *Antibodies and cell types recognized*

Antigen	Clone	Species	Source	Target Cells	Dilution	Antigen Retrieval*
MUC5AC	CLH2	Mouse	Novocastra/Leica Biosystems (Kista, Sweden)	Gastric surface cells	1:200	MW
MUC6	CLH5	M Mouse	Novocastra/Leica Biosystems	Gastric gland mucous cells	1:200	MW
GlcNAcα1→4Galβ→R	HIK1083	Mouse	Kanto Chemical, Tokyo, Japan	Gastric gland mucous cells	1:10	MW
PDX1	Polyclonal	Guinea pig	C.V. Wright, DPhil, Vanderbilt University Medical School, Nashville, TN	Pyloric and duodenal epithelial cells	1:10,000	Pascal
MUC2	Ccp58	Mouse	Novocastra/Leica Biosystems	Intestinal goblet cells	1:200	MW
CDX2	CDX2-88	Mouse	BioGenex Inc. (San Ramon, C)	Intestinal epithelial cells	1:100	MW
CPS1	OCH1E5	Mouse	Dako (Carpentaria, CA)	Small intestinal epithelial cells	1:50	MW
SATB2	SC-81376	Mouse	Santa Cruz Biotechnology (Santa Cruz, CA)	Large intestinal epithelial cells	1:50 (diluted with IMMUNO SHOT (Cosmo Bio, Tokyo, Japan)	Pascal

GlcNAcα1→4Galβ→R, N-acetylglucosaminα1→4galactoseβ→R; PDX1, pancreatic-duodenal homeobox 1; CDX2, caudal homeobox 2; CPS1, carbamoyl phosphate synthetase 1, SATB2, special AT-rich sequence-binding protein 2.
* Microwaving (MW; 650 W) for 25 min in 10 mmol/L Tris-HCl buffer containing 1 mmol EDTA-2Na, pH 8.6; Pascal pressurized heating chamber (Dako) at 125°C for 30 sec in 10 mmol/L citrate buffer, pH 6.0; Pascal pressurized heating chamber (Dako) at 125°C for 3 min in Histofine antigen-retrieval solution, pH 9.0 (Nichirei).

antibody before using the Histofine detection system. Prior to immunostaining, antigen retrieval was performed using the following methods; for MUC5AC, MUC6, GlcNAcα1→4Galβ→R, MUC2, CDX2, and CPS1, samples were heated in a microwave at 650W for 25 min (in 0.01 M Tris-HCl buffer containing 1 mM EDTA-2Na, pH 8.6), for PDX, samples were heated in a Pascal pressurized heating chamber (Dako) at 125°C for 30 sec (in 10 mmol/L citrate buffer, pH 6.0) and for SATB2, samples were heated in a Pascal pressurized heating chamber (Dako) at 125°C for 3 min (in Histofine antigen-retrieval solution, pH 9.0, Nichirei). Details of antibodies and antigen retrieval methods used in this study are summarized in Table 1. Gastric mucosa samples, duodenal mucosa samples, and colon mucosa samples were used as positive controls for differentiation markers for gastric mucous cells, small intestinal epithelial cells, and large intestinal epithelial cells, respectively. Negative control samples were obtained by omitting incubation with primary antibodies.

Evaluation of immunostaining

Immunoreactivity for various markers was estimated semi-quantitatively according to the following scale; 0 (negative or <5%), 1 (focal, <1/3 of positive cells), 2 (multifocal, 1/3–2/3 of positive cells), or 3 (diffuse, >2/3 positive cells). Staining scores are non-parametric and are thus expressed as a median score with the interquartile range rather than mean values.

Phenotypic classification

As previously reported, MUC5AC is diffusely expressed in IPMNs [5, 13, 42]. The immunophenotypic classification of IPMNs was based on MUC2 expression as follows; gastric predominant (gastric type) (<1/3 positive cells), predominant intestinal type (intestinal type) (>2/3 positive cells), or mixed intestinal and gastric type (1/3–2/3 positive cells).

Statistical evaluation

Statistical analysis was performed using the Fisher's exact probability test (2×2 contingency tables) and the Mann-Whitney U test. A P value below 0.05 was considered significant.

III. Results

Clinical characteristics of IPMN types

Seventeen of all 33 IPMN cases (51.5%) were categorized as gastric type, eight cases (24.2%) were intestinal type and eight cases (24.2%) were mixed gastric and intestinal type (Table 2). No significant differences in terms of age and sex were observed between the different IPMN types (Table 2).

Morphological characteristics of IPMN types

Gastric type

These exhibited gastric foveola-like papillae lined by columnar cells, scattered goblet cells, clusters of pyloric-type glands lined by cuboidal cells at the base of foveola-like papillae (Fig. 1a), or flat epithelium lined by columnar or cuboidal cells (Fig. 2a). A papillary intraluminal growth pattern with protrusion into the pancreatic duct was observed in the main pancreatic duct in five cases, but not in the branch pancreatic duct. These lesions were composed of surface papillary proliferations lined by columnar cells and narrow or cystically dilated glandular structures lined by cuboidal to columnar epithelial cells.

Table 2. *Clinicopathological characters of different immunophenotypes of IPMNs*

	Gastric immunophenotype	Intestinal immunophenotype	Mixed gastric and intestinal immunophenotype
No. of cases	17	8	8
Age, y			
Range	46–81	58–77	56–72
Mean±SEM	69.2±2.1	66.4±2.5	64.8±2.5
Sex, no. (%)			
Male	11 (64.7)	4 (50.0)	7 (87.5)
Female	6 (35.3)	4 (50.0)	1 (12.5)
Major localization[#,]*, no. (%)			
Main duct	3 (17.6)	5 (62.5)	0 (0)
Branch duct	14 (82.4)	3 (37.5)	8 (100)
Intraluminal nodular growth, no. (%)	5 (29.4)	4 (50.0)	3 (37.5)
Pyloric gland-like structure[##,]**, no. (%)	17 (100)	0 (0)	7 (87.5)
Atrophy of the parenchyma[##,]*, no. (%)	1 (5.9)	5 (62.5)	0 (0)
Histological grade[##,]**, no. (%)			
Low	15 (88.2)	1 (12.5)	7 (87.5)
High	2 (11.8)	7 (87.5)	1 (12.5)
Associated invasive carcinoma, no. (%)	1 (5.9)	1 (12.5)	1 (12.5)

SEM, standard error of the mean.
Data were analyzed using the Fisher's exact probability test (2×2 tables).
(gastric type vs intestinal type: [#] $P<0.05$, [##] $P<0.01$; intestinal type vs mixed type: * $P<0.05$; ** $P<0.01$).

Intestinal type

These exhibited villus-like papillae (long finger-like projections) lined by mucin-rich columnar cells with intestinal crypt-like structures at the base of the villus structure without a pyloric gland-like structure (Fig. 3a) or a low papillary configuration (Fig. 4a). A papillary, intraluminal growth pattern was observed in the main pancreatic duct in one case and in the branched duct in three cases.

Mixed gastric and intestinal type

Histologically, two subtypes were recognized: collision type (seven cases) (Fig. 5a) and composite type (one case) (Fig. 6a). The collision type comprised a gastric type IPMN component and an intestinal type IPMN component with an interface between the two adjacent components (Fig. 5a). The composite type exhibited a close admixture of gastric-type IPMN features and intestinal-type IPMN features (Fig. 6a).

Histological differences between gastric, intestinal, and mixed gastric and intestinal type IPMN

The majority of gastric type (82%) and all mixed type IPMN were of branch-duct type, whereas the intestinal type was predominantly of main-duct type (62.5%). Pyloric gland-like structures were frequently observed in gastric and mixed types, but not in intestinal type IPMN. Parenchymal atrophy with extensive fibrosis was observed in tissues surrounding dilated ducts with tumor cell extension and was a common feature in the intestinal type, but not in gastric and mixed types. The gastric type IPMNs (88.2%) and mixed gastric and intestinal type IPMNs (87.5%) were generally low-grade dysplasia, whereas, the intestinal type IPMNs (87.5%) were generally high-grade dysplasia.

There was a significant difference between gastric and intestinal type and mixed and intestinal type IPMNs in terms of major location, pyloric gland-like structure, parenchymal atrophy, and histological grade. There was no significant difference between gastric and mixed type. No significant differences were observed among the three IPMN types in terms of mural nodule and invasive carcinoma. The results of these histological differences are summarized in Table 2.

Expression profile of mucins and cell-lineage markers in normal gastrointestinal tract and pancreatic tissue and IPMNs based on immunohistochemical staining

Normal gastrointestinal tracts

Histological sections of normal gastrointestinal tract tissue are shown in Figures 7a, 8a and 9a. As previously reported, MUC5AC was expressed in gastric surface mucous cells (Fig. 7b) [6]. MUC6 and GlcNAcα1→ 4Galβ →R were expressed in pyloric gland cells (Fig. 7c) and Brunner gland cells [6, 25, 30]. PDX1 was localized in the nuclei of gastric surface mucous cells, pyloric gland cells (Fig. 7d), and epithelial cells of the duodenum (Fig. 8b) [16, 27, 33]. MUC2 was expressed in the goblet cells of duodenum (Fig. 8c) and colon (Fig. 9c). CDX2 was localized in the nuclei of epithelial cells of villi and crypts of the duodenum (Fig. 8d) and colonic epithelial cells (Fig. 9d) [4, 9]. CPS1 was expressed in the epithelial cells of villi and crypts of the duodenum (Fig. 8e) but not in gastric and colonic epithelial cells (Fig. 9e) [8, 18]. SATB2 was expressed in the nuclei of colonic epithelial cells (Fig. 9f), but not in gastric and duodenal epithelial cells (Fig. 8f) [19].

Normal pancreatic tissues

Histological sections of normal pancreatic tissues are

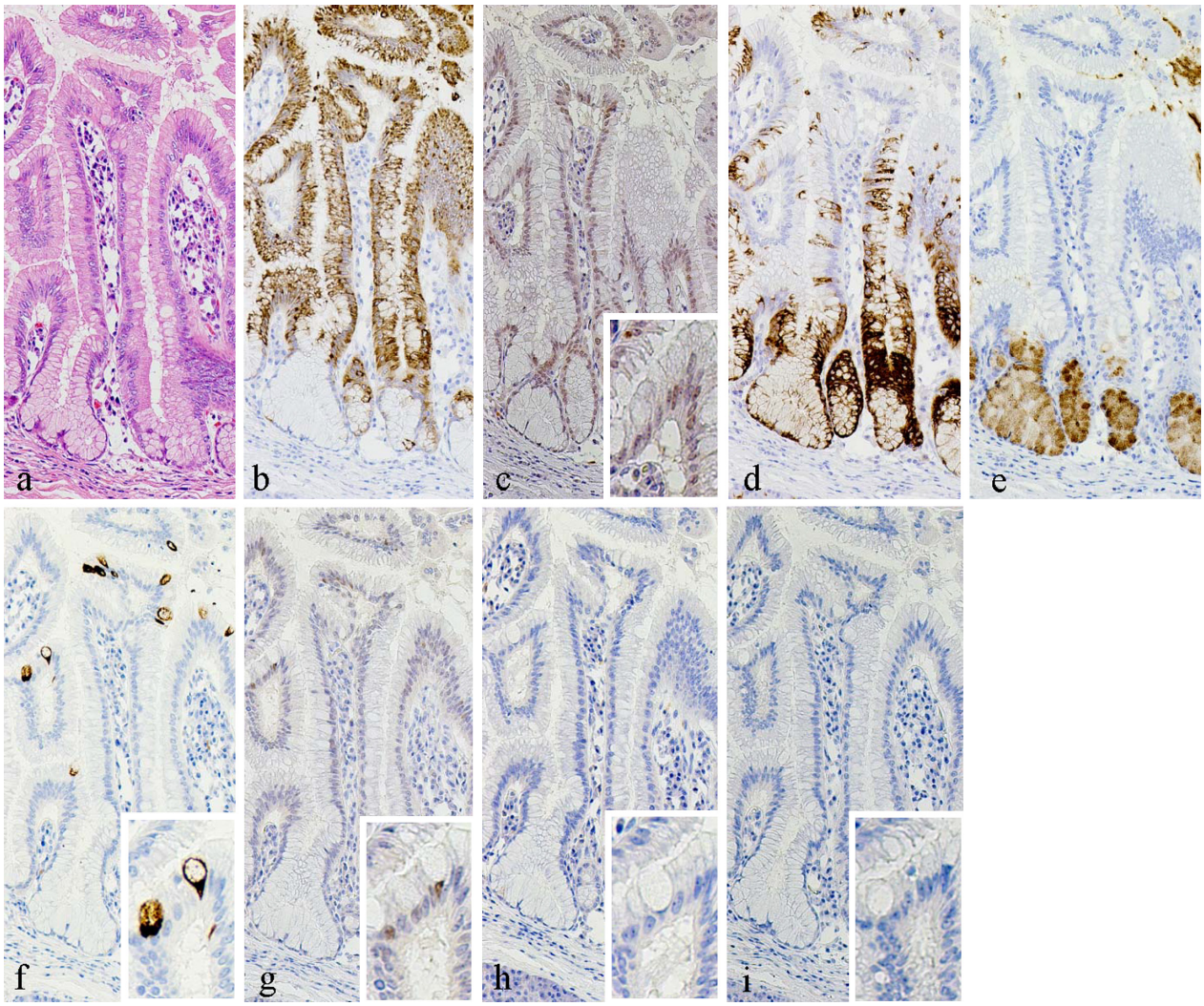

Fig. 1. Gastric type intraductal papillary mucinous neoplasm (IPMN) characterized by gastric foveola-like papillae lined by columnar cells and pyloric gland-like mucous glands lined by cuboidal cells at the base of foveola-like papillae (**a:** Hematoxylin and eosin (HE) staining). MUC5AC expression is detected throughout the lesion, however, reduced expression is observed in pyloric gland-like mucous glands (**b:** MUC5AC). PDX1 is diffusely expressed in the nuclei of neoplastic cells throughout the lesion (inset) (**c:** PDX1). MUC6 is expressed with an increasing expression gradient towards the pyloric gland-like structure (**d:** MUC6). GlcNAcα1→4Galβ→R is expressed in tumor cells of pyloric gland-like structures (**e:** GlcNAcα1→4Galβ→R). Interspersed goblet cells are positive for MUC2 (inset) (**f:** MUC2), CDX2 (inset) (**g:** CDX2). Neoplastic cells are negative for CPS1 (inset) (**h:** CPS1) and for SATB2 (inset) (**i:** SATB2).

shown in Figures 10a and 11a. PDX1 was expressed in the nuclei of epithelial cells of intercalated and intralobular ducts (Fig. 10b) and periductal glands (Fig. 11b). MUC6 expression was observed in acinal (Fig. 10c), intercalated and intralobular ducts (Fig. 10c) and in periductal gland cells (Fig. 11c). HIK1083-reactive mucin was detected in periductal gland cells (Fig. 11d), but not in acinal and ductal cells (Fig. 9d and 10d). CDX2-weakly positive cells were dispersed in intercalated and intralobular ducts. MUC5AC, MUC2, SP1, and SATB2 were not expressed in normal pancreatic tissues.

IPMNs

In gastric type IPMN, MUC5AC was diffusely expressed in all 17 cases. The expression of MUC5AC was decreased in the basal portion of the papillary structures or in pyloric gland-like structures (Fig. 1b). PDX1 was expressed in all cases, with focal expression in three cases (17.6%), multifocal expression in one case (5.9%), and diffuse expression in the remaining 13 cases (76.5%) (Fig. 1c). MUC6 was expressed in all cases with multifocal expression observed in one case (5.9%), and diffuse expression observed in 16 cases (94.1%). HIK1083-reactive mucin was detected in all cases with focal expression observed in one (5.9%), multifocal expression observed in four cases (23.5%), and diffuse expression observed in 12 cases (70.6%). MUC6 and HIK1083-reactive mucin were expressed with an increasing expression gradient towards the papillary base or pyloric gland-like structure (Fig. 1d and 1e). In flat lesions, MUC5AC expression was inversely related to the expression of MUC6 and HIK1083-reactive mucin (Fig. 2b, 2d and 2e). MUC2 and CDX2-positive

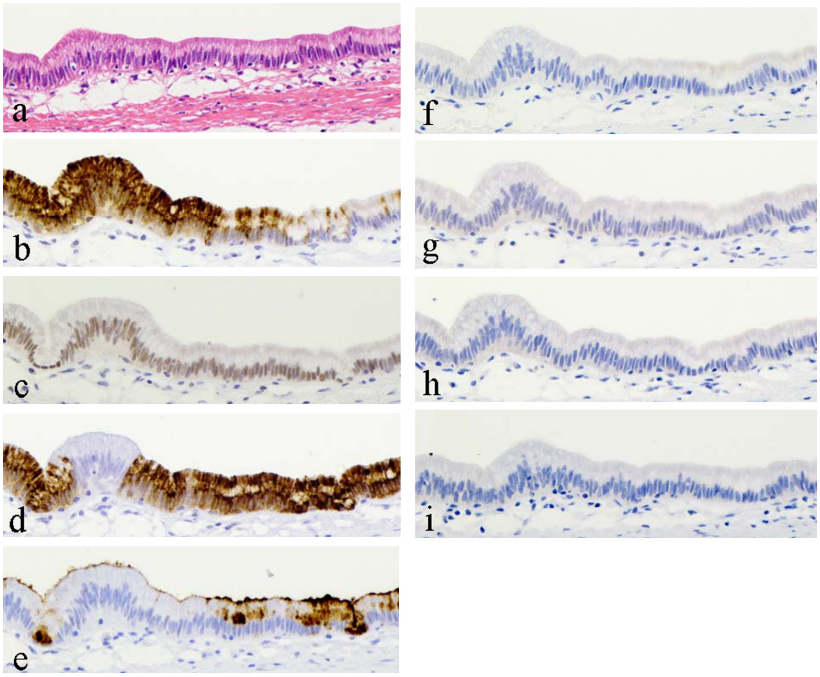

Fig. 2. Gastric type IPMN demonstrating flat epithelium lined by columnar or cuboidal cells (**a:** HE). MUC5AC and PDX1 are diffusely expressed in epithelial cells (**b:** MUC5AC, **c:** PDX1). MUC5AC expression is inversely related to MUC6 (**d:** MUC6) and GlcNAcα1→4Galβ→R expression (**e:** GlcNAcα1→4Galβ→R). MUC2, CDX2, CPS1, and SATB2 are not expressed (**f:** MUC2, **g:** CDX2, **h:** CPS1, **i:** SATB2).

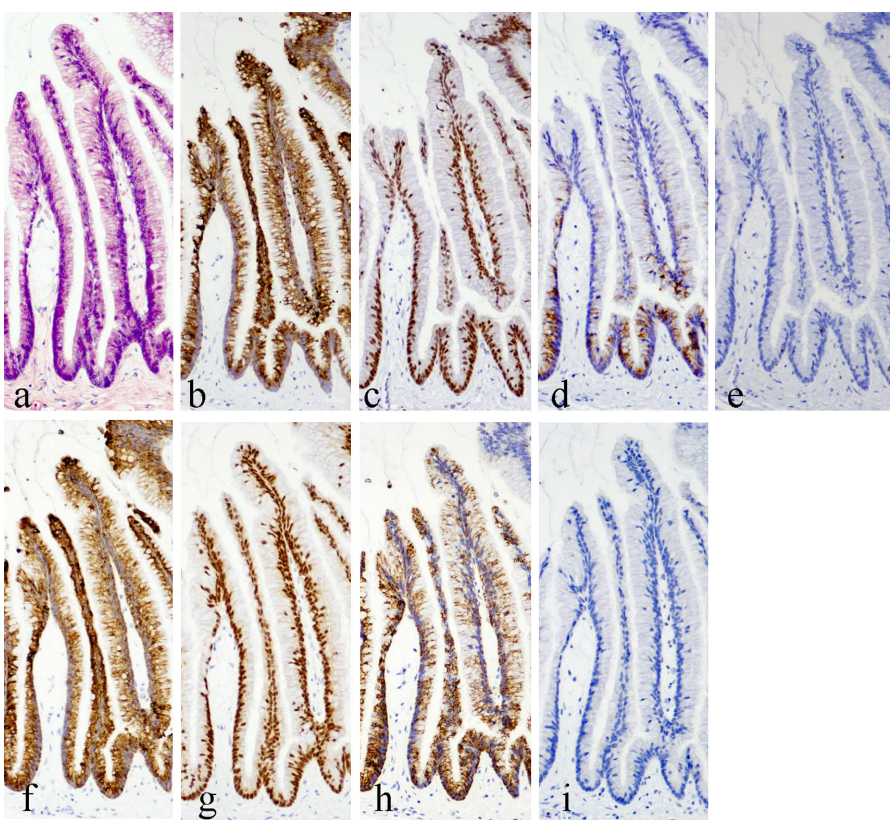

Fig. 3. Intestinal type IPMN showing villus-like papillae (long finger-like projections) lined by mucin-rich columnar cells with intestinal crypt-like structures at the base of the villus structure without pyloric glands-like structures (**a:** HE). MUC5AC and PDX1 are expressed throughout the lesion (**b:** MUC5AC, **c:** PDX1). MUC6 is expressed in tumor cells at crypt-like structures (**d:** MUC6), whereas GlcNAcα1→4Galβ→R is not expressed (**e:** GlcNAcα1→4Galβ→R). MUC2, CDX2 and CPS1 are diffusely expressed (**f:** MUC2, **g:** CDX2, **h:** CPS1). No staining for SATB2 is observed (**i:** SATB2).

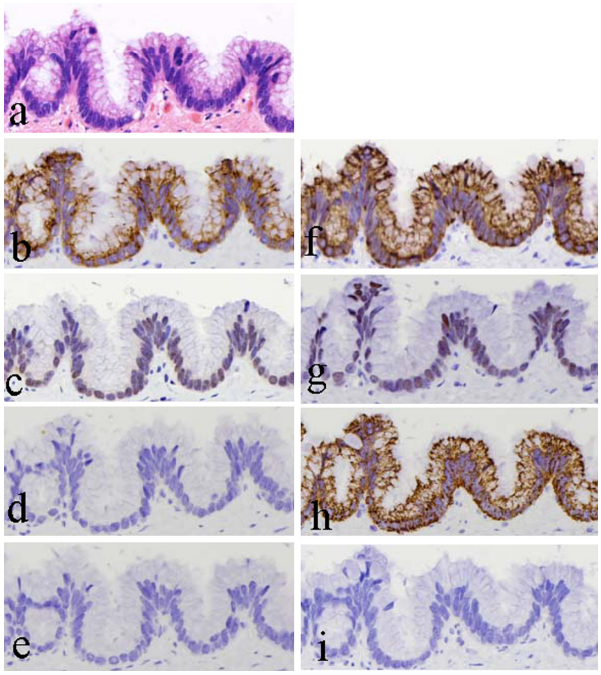

Fig. 4. Intestinal type IPMN showing low papillary configuration (**a:** HE). MUC5AC and PDX1 are diffusely expressed in epithelial cells (**b:** MUC5AC, **c:** PDX1). MUC6 and GlcNAcα1→4Galβ→R are not expressed (**d:** MUC6, **e:** GlcNAcα1→4Galβ→R). MUC2, CDX2 and CPS1 are diffusely expressed (**f:** MUC2, **g:** CDX2, **h:** CPS1). SATB2 is not expressed (**i:** SATB2).

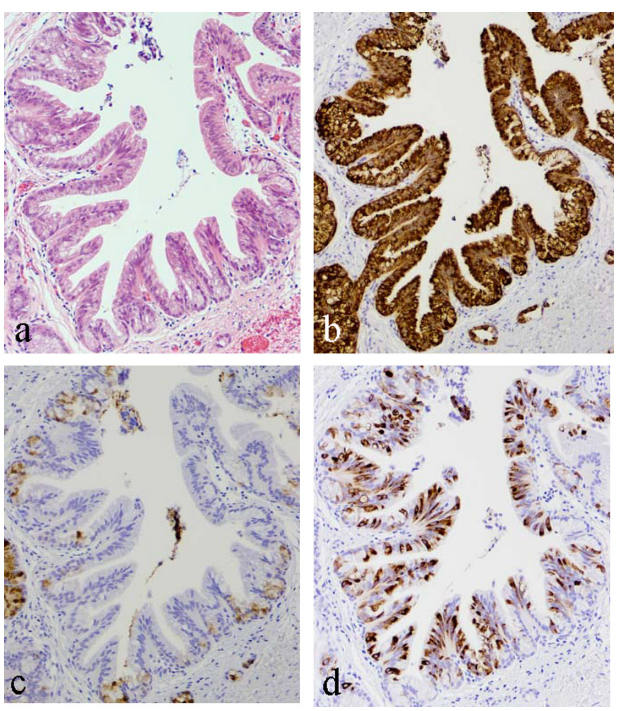

Fig. 6. Composite type of mixed gastric and intestinal type IPMN (**a:** HE). MUC5AC is diffusely expressed in epithelial cells (**b:** MUC5AC). The composite type exhibits pyloric gland-like structures composed of GlcNAcα1→4Galβ→R-positive mucous cells at the base (**c:** GlcNAcα1→4Galβ→R) and papillary or villous-like structures lined by MUC2-positive cells (**d:** MUC2).

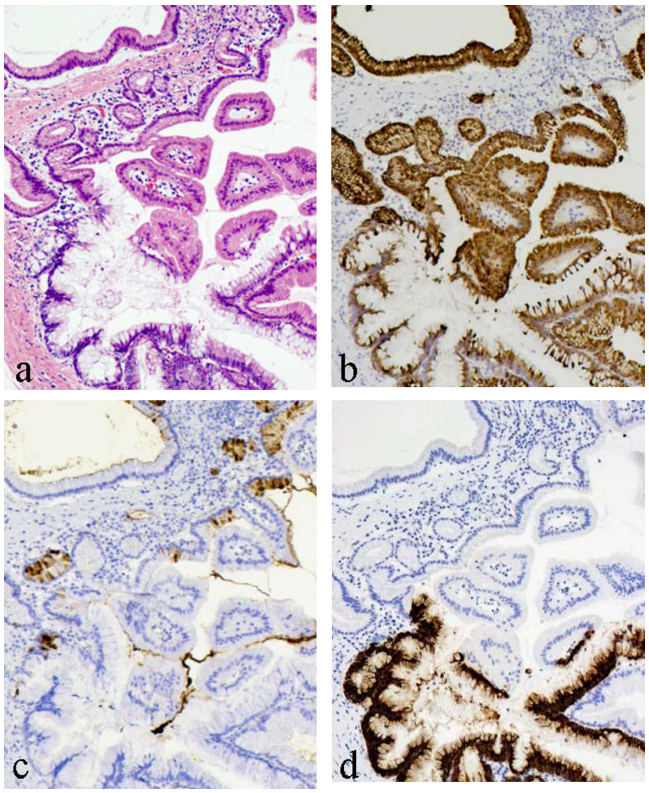

Fig. 5. Collision type of mixed gastric and intestinal type IPMN (**a:** HE). MUC5AC is diffusely expressed in epithelial cells (**b:** MUC5AC). The collision type comprises two components: GlcNAcα1→4Galβ→R-positive (**c:** GlcNAcα1→4Galβ→R) and/or MUC2-negative (**d:** MUC2) gastric and MUC2-positive (**d:** MUC2) intestinal component with an interface between the two adjacent components.

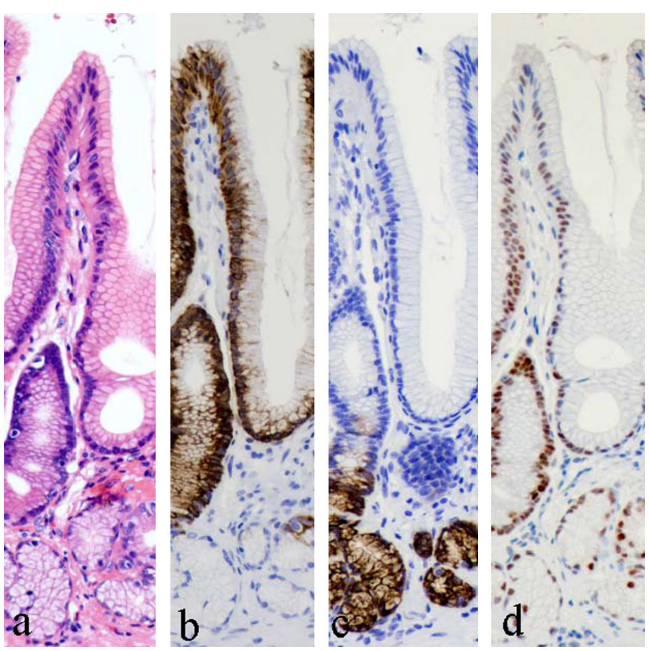

Fig. 7. Normal gastric pyloric mucosa (**a:** HE). MUC5AC, MUC6 and PDX1 are expressed in surface mucous cells (**b:** MUC5AC), in pyloric gland cells (**c:** MUC6), and in the nuclei of pyloric epithelial cells (**d:** PDX1), respectively.

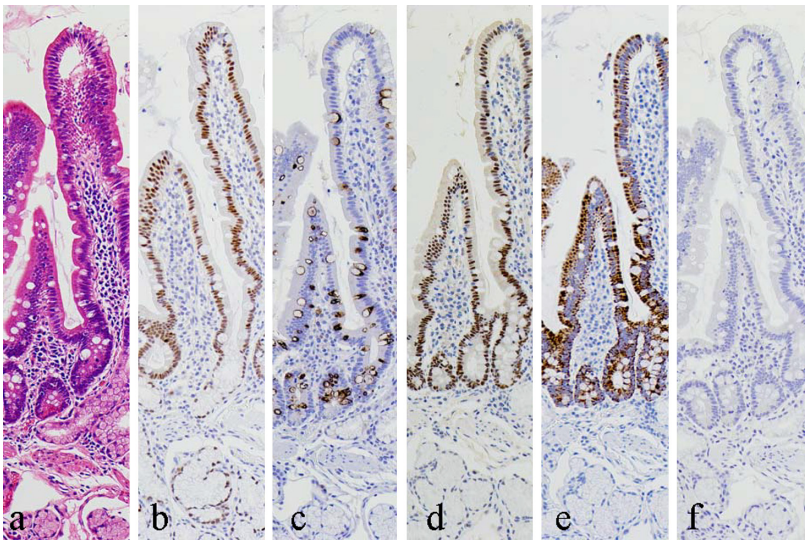

Fig. 8. Normal duodenal mucosa (**a:** HE). PDX1, MUC2, CDX2, and CPS1 are expressed in the nuclei of duodenal epithelial cells (**b:** PDX1), in goblet cells (**c:** MUC2), in the nuclei of duodenal villi and crypt cells (**d:** CDX2), in duodenal villi and crypt cells (**e:** CPS1). SATB2 is not expressed (**f:** SATB2).

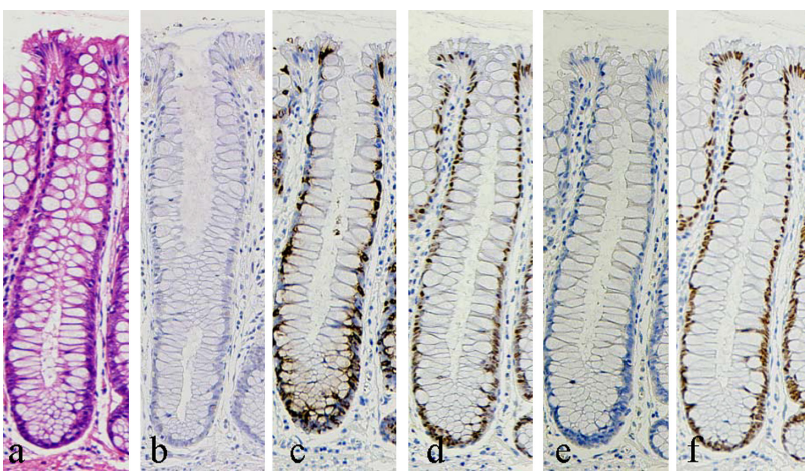

Fig. 9. Normal colon mucosa (**a:** HE). PDX1 is not expressed (**b:** PDX1). MUC2 and CDX2 are expressed in colonic goblet cells (**c:** MUC2) and in the nuclei of colonic epithelial cells (**d:** CDX2), respectively. CPS1 is not expressed (**e:** CPS1). SATB2 is expressed in the nuclei of colonic epithelial cells (**f:** SATB2).

cells were *interspersed* or exhibit focal expression in six cases (35.3%) and three cases (17.6%), respectively. These MUC2 and CDX2-positive cells were observed in papillary structures (Fig. 1f and 1g), but not in flat lesions (Fig. 2f and 2g). CPS1 expression was generally absent (Fig. 1h and 2h), although focal expression was detected in one case (5.9%).

In intestinal type IPMN, MUC5AC were expressed in all eight cases with diffuse expression observed in seven cases (87.5%) and multifocal expression in one case (12.5%) (Fig. 3b and 4b). PDX1 was diffusely expressed in all cases (Fig. 3c and 4c). MUC6 was focally expressed in seven cases (87.5%) at the base of villus-like papilla or crypt-like structures (Fig. 3d), but not in low papillary lesions (Fig. 4d). In contrast, HIK1083-reactive cells were not detected (Fig. 3e and 4e). MUC2 was diffusely expressed in all cases (Fig. 3f and 4f). CDX2 was expressed in all cases with multifocal expression observed in two cases (25%) and diffuse expression in six cases (75%) (Fig. 3g and 4g). CPS1 was expressed in six cases (75%) with focal expression in one case (12.5%), multifocal expression in two cases (25%), and diffuse expression in three cases (37.5%) (Fig. 3h and 4h).

Mixed gastric and intestinal type IPMN was best characterized by the pattern of distribution of two components: MUC2-negative and/or GlcNAcα1→4Galβ→R-positive gastric and MUC2-positive intestinal components (Fig. 5). The collision type exhibited an interface between the two adjacent components (Fig. 5), whereas the composite type exhibited a close admixture of the two components (Fig. 6).

SATB2 expression was not detected in any IPMNs (Fig. 1i, 2i, 3i and 4i).

Expression of MUC6 was higher in gastric and in mixed type than in intestinal type IPMN. Staining scores

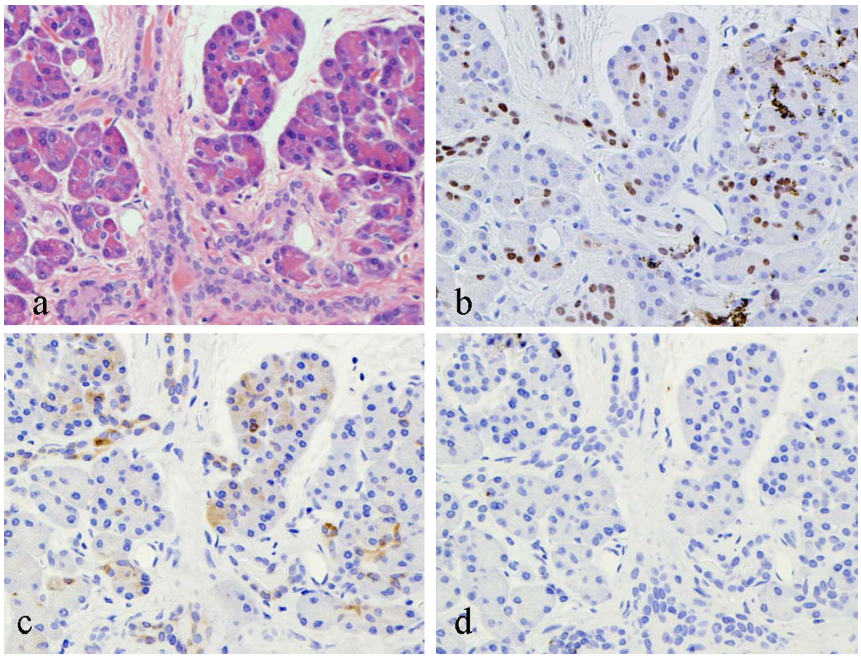

Fig. 10. Normal pancreatic tissue (**a:** HE). PDX1 is expressed in the nuclei of epithelial cells of intercalated ducts and intralobular ducts (**b:** PDX1). MUC6 is expressed in some acinal cells and in epithelial cells of intercalated ducts and intralobular ducts (**c:** MUC6). GlcNAcα1→4Galβ→R is not expressed (**d:** GlcNAcα1→4Galβ→R).

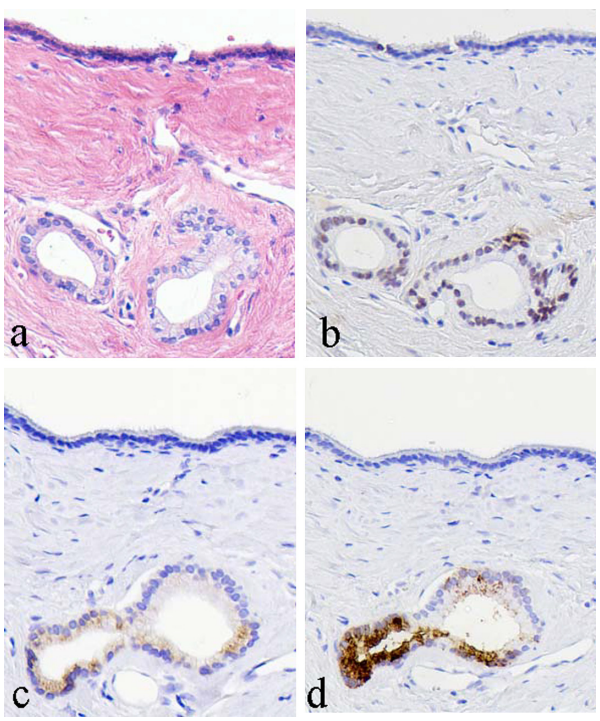

Fig. 11. Normal main pancreatic duct (**a:** HE). PDX1, MUC6 and GlcNAcα1→4Galβ→R are expressed in periductal mucous gland cells, but not in ductal epithelial cells (**b:** PDX1, **c:** MUC6, **d:** GlcNAcα1→4Galβ→R).

for MUC2 and CDX2 were higher in intestinal type than gastric and mixed type, while scores for CPS1 were higher in intestinal type than gastric type. These results are summarized in Table 3.

IV. Discussion

In this study, we have confirmed and extended our knowledge regarding immunophenotypic characteristics of gastric and intestinal IPMN, which represent the two major forms of this disease. We demonstrate that gastric pyloric and small intestinal differentiation are characteristic of gastric and intestinal type IPMN, respectively. Clinicopathological and immunophenotypic differences between these two types of IPMN favor the hypotheses that these two types of IPMN may be distinct entities with different pathogenesis.

Based on the degree of MUC2 expression, IPMNs (other than pancreatobiliary type and oncocytic type) are subdivided into three types: gastric, intestinal and mixed gastric and intestinal type. Pathological characteristics of IPMNs and the surrounding parenchyma of IPMNs of gastric and intestinal types defined in this study correspond to previously described gastric and intestinal type IPMNs [2, 3, 10, 14, 36, 39]. In keeping with previous reports [1, 13, 26, 42], MUC2 expression is a reliable marker for discriminating between the gastric type IPMN with foveola-pyloric gland and flat structures and intestinal type IPMN with the villous-crypt and low papillary structures.

In the present study, PDX-1, a transcription factor that plays an essential role in the genesis and development of the gastric antrum, duodenum, and pancreas [28, 37], was frequently expressed in the nuclei of epithelial cells of IPMNs in conjunction with aberrant expression of gastric and intestinal lineage markers. This finding is similar to immunohistochemical analyses reported by Park *et al.* [32], who also observed expression of PDX1 in normal human

Table 3. *Immunostaining of gastric-, intestinal-, and mixed gastric and intestinal-type IPMNs*

Type of IPMN	Cell lineage markers							
	Gastric surface mucous cell	Gastric gland mucous cell		Pyloric and duodenal epithelial cell	Intestinal goblet cell	Intestinal epithelial cell	Small intestinal epithelial cell	Large intestinal epithelial cell
	MUC5AC	MUC6	GlcNAcα1→4Galβ→R	PDX1	MUC2	CDX2	CPS1	SATB2
Gastric (n=17)	17 (100)/3 (3,3)[a]	17 (100)/3 (3,3)[##]	17 (100)/3 (2,3)[##]	17 (100)/3 (3,3)	6 (35.3)/0 (0,1)[##.**]	3 (17.6)/0 (0,0)[##.**]	1 (5.9)/0 (0,0)[##.**]	0 (0)/0 (0,0)
Intestinal (n=8)	8 (100)/3 (3,3)	7 (87.5)/1 (1,1)[††]	0 (0)/0 (0,0)[††]	8 (100)/3 (3,3)	8 (100)/3 (3,3)[††]	8 (100)/3 (2.75,3)[†]	6 (75)/2 (0.75,3)	0 (0)/0 (0,0)
Mixed gastric and intestinal (n=8)	8 (100)/3 (3,3)	8 (100)/3 (2.75,3)	8 (100)/2.5 (2,3)	8 (100)/3 (2.75,3)	8 (100)/2 (2,2)	8 (100)/2 (2,2)	7 (87.5)/1.5 (1,2)	0 (0)/0 (0,0)

GlcNAcα1→4Galβ→R, N-acetylglucosaminα1→4galactoseβ→R; PDX1, pancreatic-duodenal homeobox 1; CDX2, caudal homeobox 2; CPS1, carbamoyl phosphate synthetase 1; SATB2, special AT-rich sequence-binding protein 2.

[a] Data are presented as frequency (percentage) of positive specimens/median score (interquartile range).

Scores for staining were analyzed by the Mann–Whitney U-test.

(gastric type vs intestinal type: [#] P<0.05, [##] P<0.01; gastric type vs mixed type: * P<0.05, ** P<0.01; intestinal type vs mixed type: [†] P<0.05, [††] P<0.01).

pancreatic ductal epithelium and in numerous pancreatic neoplasms including IPMNs [32].

In gastric type IPMN, frequent expression of PDX1 in conjunction with MUC5AC (gastric surface mucous cell-type mucin), MUC6 (gastric gland mucous cell-type mucin) and HIK1083-reactive mucin (gastric gland mucous cell-type mucin), may indicate differentiation towards gastric pyloric mucosa in gastric-type IPMN. This finding is in keeping with a previous report demonstrating that pancreatic ductal mucinous lesions express pepsinogen II, which is produced in gastric gland mucous cells (cardiac gland cells, mucous neck cells, and pyloric gland cells) and chief cells, but not pepsinogen I, which is limited to gastric mucous neck cells and chief cells [35]. Interestingly, several previous studies have reported similar ectopic expression of PDX1 in different lesions exhibiting gastric pyloric phenotype [16, 21, 27, 31, 33]. In contrast, an independent study reported no immunohistochemical detection of PDX1 in normal human gastric pyloric epithelium [32]. This discrepancy in PDX1 expression in normal gastric pyloric epithelium may be due to differences in the specificity and sensitivity of antibodies used in these different studies. Further investigation is necessary to explain the discrepancy in PDX1 expression in normal gastric pyloric epithelium.

Conversely, intestinal type IPMN expressed gastric (MUC5AC+/MUC6±) and small intestinal phenotype profiles (MUC2+/CDX2+/CPS1+/SATB2–). Further, frequent expression of PDX1 in conjunction with these intestinal markers in intestinal IPMNs may indicate differentiation towards duodenal villous-crypt epithelium in this IPMN type. The finding of small intestinal phenotype expression in intestinal IPMN in the present study is consistent with previous studies by Sessa *et al.* [35], which demonstrated no expression of CAR-5, a mucin-like antigen expressed in the colorectal epithelium, in any hyperplastic or metaplastic lesions in the pancreatic duct and pancreatic mucinous cystadenocarcinomas. In contrast with these studies, a previous histochemical study reported expression of 8-O-acetylneuraminic acid, a histochemical marker of large intestinal goblet cells, in intraductal papillary neoplasms [20] corresponding to intestinal type IPMNs. On the basis of these discrepancies, further studies are required to clarify the expression of colonic mucin in IPMNs between the studies remains to be clarified.

GlcNAcα1→4Galβ→R, recognized by the HIK1083 monoclonal antibody, is a sugar residue bound to MUC6 in normal gastric gland mucous cells [44], and is a more reliable marker for discriminating gastric-type from intestinal-type IPMN compared with MUC6. In the present study, both MUC6 and GlcNAcα1→4Galβ→R were expressed in papillary bases, pyloric gland-like structures and flat lesions in gastric-type IPMNs. MUC6 was expressed to various degrees in villus-like papilla in intestinal type IPMNs, similar to that reported in other studies [5, 24, 43]; however, GlcNAcα1→4Galβ→R was not expressed in any intestinal-type IPMNs. This finding is in agreement with a previous histochemical study by Matsuzawa *et al.* [20], which reported that mucin-producing tumors of the pancreas resembling colonic villous adenoma, lacked pyloric gland-like structures and class III mucin, and were therefore designated as intestinal-type tumors. Class III mucin is defined by paradoxical concanavalin A staining [17] and its distribution coincides with GlcNAcα1→4Galβ→R in normal, metaplastic, and neoplastic human tissues including IPMN and pancreatic ductal adenocarcinoma [30].

In the present study, MUC5AC was expressed in all IPMNs examined but not in normal pancreatic tissues, which is in accordance with results obtained in other studies [1, 5, 13, 26, 42]. The mechanisms responsible for the aberrant MUC5AC expression in IPMNs are unknown. MUC5AC may be induced in stem cells by specific transcription factors or changes in gene methylation status and

histone H3 modification, in keeping with studies reporting aberrant MUC5AC expression in various cancer cell lines (pancreas, colon, breast, and lung) [41]. The mechanisms underlying the aberrant expression of MUC5AC in IPMNs requires further investigation.

To date, two opposing hypotheses have been proposed to explain the relationship between gastric-type IPMN and intestinal-type IPMN: the conversion of the former into the latter and the independent neoplastic pathway. In support of the conversion theory, MUC2 expression is scattered or focally distributed in gastric type IPMNs, in contrast to the diffuse expression observed in intestinal type IPMNs, in conjunction with diffuse MUC5AC expression in both gastric and intestinal types. Further, gradual histological transition from gastric-type IPMN into intestinal-type IPMN has been previously reported [34]. Thus, the term 'null cell-type IPMN' has been proposed for gastric type IPMN [1]. The independent neoplastic pathway hypothesis is supported by studies demonstrating differences in the anatomic distribution and the manner of spread along the pancreatic ductal system between gastric and intestinal type IPMNs [5, 20]. This hypothesis was confirmed in our study, where gastric type was characterized by branch duct-type lesions often associated with pyloric gland-like structures, whereas the intestinal type was characterized by main duct-type lesions often associated with atrophy and fibrosis of the surrounding parenchyma. The abrupt transition between the two IPMN types previously reported in mixed-type IPMNs [26], was also observed in our study. In addition, we observed no significant differences in age between these three IPMN subtypes. In our immunohistochemical analyses, HIK1083-reactive mucin was observed in gastric and mixed phenotype, but not in intestinal type IPMN. Further, genetically, distinct mechanisms of pathogenesis underlying these two types of IPMN have been reported with KRAS-ERK signal activation frequently observed in gastric-type IPMN, whereas BMP-SMAD signal activation frequently observed in intestinal-type IPMN. It should be noted, however, that some cases exhibited both KRAS-ERK and BMP-SMAD activation, suggesting the presence of overlapping cases [22].

In summary, our study re-evaluates the phenotypic characteristics in two major types of IPMN: gastric-type and intestinal-type. We demonstrate that gastric pyloric and small intestinal differentiation are characteristic of gastric and intestinal type IPMN, respectively. Our findings support the hypothesis that these two IPMN types may have different underlying modes of pathogenesis. This conclusion is reinforced by our observations of 1) no difference in ages between the two IPMN types, 2) differences in the predominant localization and in the manner of spreading along the pancreatic ductal system between the two IPMN types, 3) frequent abrupt transition between the two IPMN types in mixed-type IPMN, and 4) expression of HIK1083-reactive mucin in gastric-type IPMN but not in intestinal type IPMN.

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