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Mucin expression profile in pancreatic cancer and the precursor lesions

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Abstract
In this review article, we demonstrate the mucin expression profile in normal tissue, invasive ductal carcinoma (IDC), two subtypes of intraductal papillary–mucinous neoplasm (IPMN dark cell type and IPMN clear cell type), pancreatic intraepithelial neoplasia (PanIN), and mucinous cystic neoplasm (MCN) of the pancreas. In MUC1, there are various glycoforms, such as poorly glycosylated MUC1, sialylated MUC1, and fully glycosylated MUC1. IDCs showed high expression of all the glycoforms of MUC1. IPMNs dark cell type showed no expression or low expression of all the glycoforms of MUC1. IPMNs clear cell type showed low expression of poorly glycosylated MUC1, but expression of sialylated MUC1 and fully glycosylated MUC1. Expression of MUC2 was negative in IDCs, high in IPMNs dark cell type and low in IPMNs clear cell type. MUC5AC was highly expressed in IDCs, IPMNs dark cell type, and IPMNs clear cell type. MUC6 expression was higher in IPMNs clear cell type than in IDCs and IPMNs dark cell type. Our recent study demonstrated that high expression of MUC4 in IDCs is correlated with a poor outcome for patients. In PanINs, expression of both MUC5AC and MUC6 are an early event, whereas upregulation of MUC1 is a late event. MCNs do not look as if they will show a specific mucin expression profile according to the literature review.

Key words Pancreas · Invasive ductal carcinoma · Intraductal–papillary mucinous neoplasm · Pancreatic intraepithelial neoplasia · Mucinous cystic neoplasm · Mucin

Introduction

In Japan, invasive ductal carcinoma of the pancreas (IDC) is the fifth leading cause of cancer death in men, and the sixth leading cause of cancer death in women. The incidence rate in Japan is close to the level found in Europe or the United States when the age-adjusted incidence rates are compared. Patients with IDC still show a poor clinical outcome, in spite improvements in the diagnosis and treatment methods. The overall 5-year survival rate for all patients, with or without pancreatectomy, after diagnosis is 9.7% in Japan. On the other hand, the patients with a successful resection of IDCs at an early stage (Stage Ia) have a 39.9% 5-year survival rate.[1] However, most patients with IDCs are diagnosed in the advanced stages because of the anatomical location of the pancreas, lack of specific symptoms, infiltration to the surrounding organs, or distant metastasis even from a small primary tumor less than 2 cm in diameter.

Mucins are high molecular weight glycoproteins with oligosaccharides attached to serine or threonine residues of the mucin core protein backbone by *O*-glycosidic linkages, which are produced by various epithelial cells. Core proteins for human mucins (MUC1-MUC9, MUC11-13, MUC15-20) have been identified recently.[2–13] Mucins are categorized into membrane-associated mucins (MUC1, MUC3 MUC4, MUC12, MUC16, and MUC17), gel-forming mucins (MUC2, MUC5AC, MUC5B, and MUC6), and soluble mucin (MUC7).[14]

Our series of immunohistochemical studies for mucin expression in various human tumors, including pancreatic tumors, have demonstrated that the expression of MUC1 mucin (membrane mucin) is related to an invasive proliferation of tumors and/or a poor outcome for the patient.[15] On the other hand, the expression of MUC2 mucin (intestinal-type secretory mucin) is related to noninvasive proliferation of tumors and/or a favorable outcome for the patient.[2,16–22]

In this review article, we demonstrate the expression of several mucins, i.e., MUC1 (pan-epithelial membrane mucin), MUC2 (intestinal-type secretory mucin), MUC5AC (gastric surface mucous epithelial mucin), and MUC6 (gastric pyloric glandular mucin) in normal

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tissue, tumors, and the precursor lesions of the pancreas. The distribution of mucins in normal pancreatic tissue is demonstrated first. This is followed by the expression profile of mucins in IDCs and intraductal papillary–mucinous neoplasms (IPMNs). We describe our recent study of the expression of MUC4 (respiratory epithelial mucin) expression in IDCs. The expression profiles of mucins in pancreatic intraepithelial neoplasias (PanINs) and mucinous cystic neoplasms (MCNs) are also reviewed.

Distribution of mucins in normal pancreatic tissue

In MUC1, various glycoforms of MUC1, from a poorly glycosylated form to a fully glycosylated form, are known to exist. These variations can be detected immunohistochemically using epitope-specific monoclonal antibodies. For a simple representation, "MUC1/CORE, MUC1/DF3, MUC1/MY.1E12, and MUC1/HMFG-1" were used for the MUC1 mucin antigens detected by monoclonal antibodies. The expressions of each MUC1 are summarized in Table 1 and Figs. 1 and 2. Generally, every MUC1 is expressed in the cell apices of the centroacinar cells, intercalated ducts, intralobular ducts, and focally in the interlobular ducts, but not in the main pancreatic ducts, acini, or islets.

MUC2 and MUC5AC are never expressed in normal pancreatic tissue. MUC2 expression in the perinuclear region of the goblet cells of normal intestinal mucosa, MUC5AC expression in the surface mucous cells in normal gastric mucosa, and MUC6 expression in the cytoplasm of normal gastric pyloric glands served as a positive control for their expression. MUC6 expression was observed in the periductal glands in normal pancreatic tissue, and served as an internal positive control. MUC6 was expressed in the normal ductal epithelium in 6 of 9 cases examined.[23] MUC6 was also expressed in the acini in some cases but not in other cases, although the reason for the discrepancy is unknown (unpublished data).

Table 1. MUC1 expression in normal pancreatic tissue

	MUC1			
	CORE	DF3	MY.1E12	HMFG-1
Central areas of acini	–/+	–/+	++	++
Intercalated ducts	+	+	+	+
Intralobular ducts	+	+	+	–/+
Interlobular ducts	–	–/+	–/+	–
Main pancreatic ducts	–	–	–	–

From [25], with permission

Expression profile of mucins in IDCs and IPMNs

Sixty-three cases of IPMNs were classified into two subtypes, 27 IPMNs dark cell type (IPMNs-D) composed of dark columnar cells which form villous architecture morphologically similar to colonic villous adenoma (Fig. 3A, left-hand side), and 36 IPMNs clear cell type (IPMNs-C) composed of clear columnar cells which form papillary architecture (Fig. 3B, left-hand side) by the hematoxylin and eosin finding, according to our previous reports.[24,25] Fukushima et al.[26] also reported a similar observation. In comparison with a classification by Adsay et al.,[27] IPMNs-D is the same tumor as the "intestinal type," and the histological finding of severe atypia or carcinomatous change in

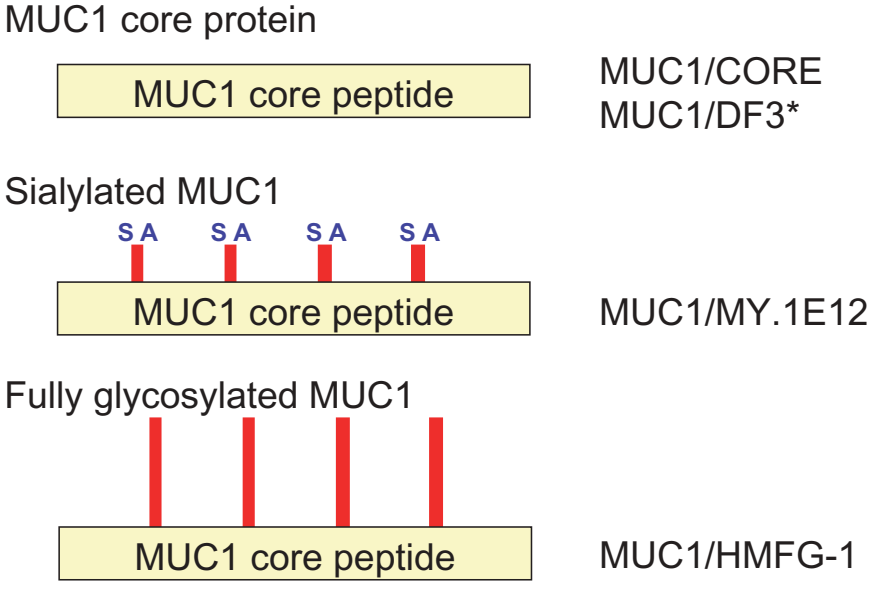

Fig. 1. Different glycoforms of MUC1 and specific antibodies. MUC1 glycoform (*left*) is recognized by various antibodies (*right*). *MUC1/DF3 basically reacts with core peptide, but sialic acid modification may enhance the affinity (Siddiqui J et al. (1988) Proc Natl Acad Sci USA 85: 2320–2323)

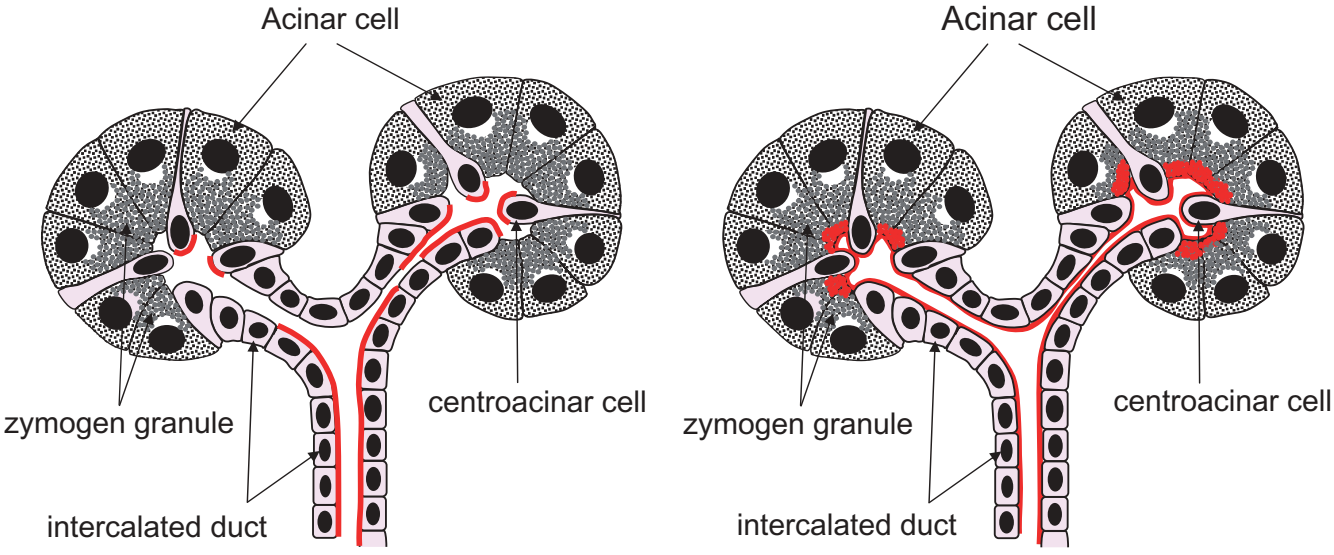

Fig. 2. Normal distribution of MUC1 at the acinar to intercalated duct area, immunohistochemically detected by four types of MUC1 antibodies recognizing different glycoform patterns. Compared with anti-MUC1 core antibodies labeling intercalated duct and centroacinar cells (**A**), antibodies detecting the sialylated or fully glycosylated form (**B**) label a wider area, including the luminal surface of acinar cells

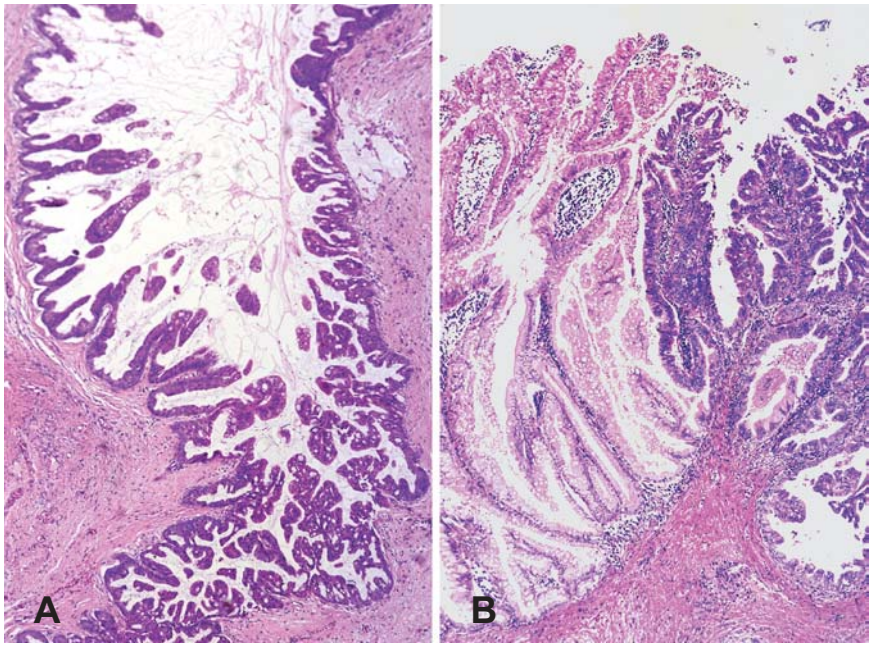

Fig. 3. In intraductal papillary–mucinous neoplasms (IPMNs), there are at least two different types: (**A**) dark-cell type (IPMNs-D), and (**B**) clear-cell type (IPMNs-C). (Hematoxylin and eosin, ×55). (From [25], with permission)

IPMNs-C is very similar to that of the "pancreatobiliary type" (Fig. 3B, right-hand side). Figure 4 shows representative mucin expression patterns in IDCs, IPMNs-D, and IPMNs-C. Figure 5 shows a comparison of the expression rates of the core proteins of each mucin (MUC1, MUC2, MUC3, MUC4, MUC5AC, and MUC6), sialyl Tn[28] and CD10[23] in normal ducts, PanINs (Fig. 6; see the description in the section "Expression profile of mucins in PanINs"), IDCs, IPMNs, and MCNs. In Fig. 5, generic alternations in normal ducts, PanINs, IDCs, IPMNs, and MCNs from various sources are also demonstrated.[28–47] In the data on IPMNs and MCNs, the cases carrying carcinoma ware not included.

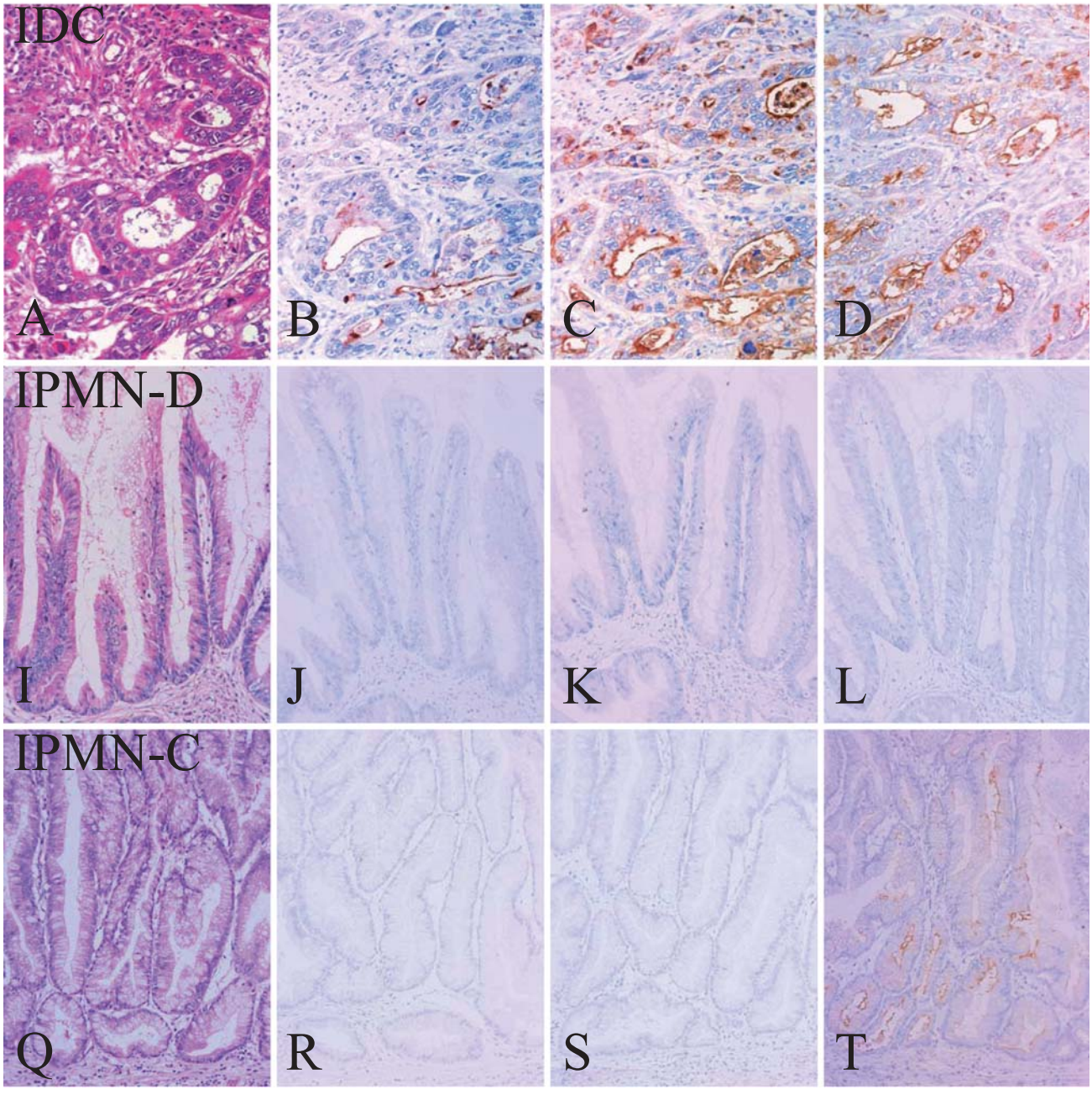

Fig. 4. Expression of MUC1, MUC2, MUC5AC, and MUC6 in invasive ductal carcinoma of the pancreas (IDC) (*upper row*), IPMN-D (*middle row*), and IPMN-C (*lower row*). Note that various glycoforms of MUC1 are expressed in the three types of tumor. Hematoxylin and eosin (**A**, **I**, **Q**); MUC1/CORE (**B**, **J**, **R**); MUC1/DF3 (**C**, **K**, **S**); MUC1/MY.1E12 (**D**, **L**, **T**); MUC1/HMFG-1 (**E**, **M**, **U**); MUC2 (**F**, **N**, **V**); MUC5AC (**G**, **O**, **W**); MUC6 (**H**, **P**, **X**). ×140 (**A**–**H**, **W**, **X**); ×70 (**I**–**V**). (From [25], with permission)

Expression of different glycoforms of MUC1 mucin (membrane-type mucin)

IDCs showed high expression rates of every MUC1 (MUC1/CORE, 66%; MUC1/DF3, 96%; MUC1/MY.1E12, 98%; MUC1/HMFG-1, 76%). IPMNs-D showed no expression or low expression rates of every MUC1 (MUC1/CORE, 0%; MUC1/DF3, 0%; MUC1/MY.1E12, 0%; MUC1/HMFG-1, 4%). IPMNs-C showed low expression rates of the under-glycosylated MUC1 (MUC1/CORE, 6%; MUC1/DF3, 3%), but considerably higher expression rates of sialylated or fully glycosylated MUC1 (MUC1/MY.1E12, 41%; MUC1/HMFG-1, 69%).

The expression rates of MUC1/CORE and MUC1/DF3 showed no significant difference between IPMNs-

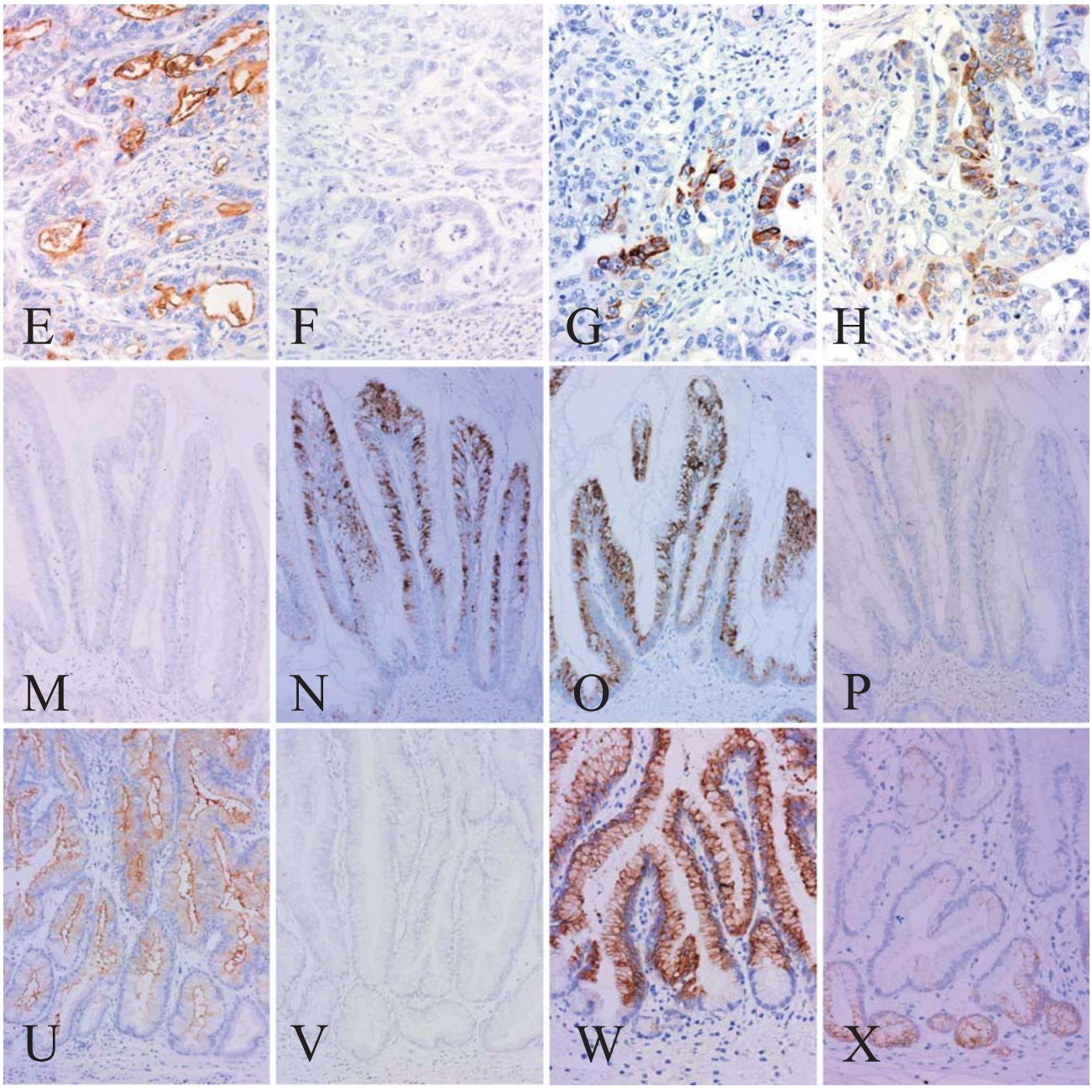

Fig. 4. *Continued*

D and IPMNs-C, although those in IPMNs-D and IPMNs-C were significantly lower than those in IDCs ($P < 0.0001$).

The expression rates of MUC1/MY.1E12 and MUC1/HMFG-1 in IPMNs-D were significantly lower than those in IPMNs-C ($P < 0.0001$), and also significantly lower than those in IDCs ($P < 0.0001$). There was no significant difference in the expression rate of MUC1/HMFG-1 between IPMNs-C and IDCs.

In summary, all the glycoforms of MUC1 showed high expression in IDCs. MUC1/CORE and MUC1/DF3 were rarely expressed in both IPMNs-D and IPMNs-C, whereas MUC1/MY.1E12 and MUC1/HMFG-1 showed low expression in IPMNs-D but high expression in IPMNs-C.

For the glycosylation status of MUC1 mucins in carcinoma tissue, a previous study stressed that MUC1 expressed in breast carcinomas is poorly glycosylated in the MUC1 mucin, whereas normal breast tissue shows little or no expression of the MUC1 mucin core peptide.[48] This phenomenon is explained in part by the finding that MUC1 core peptide epitopes are masked

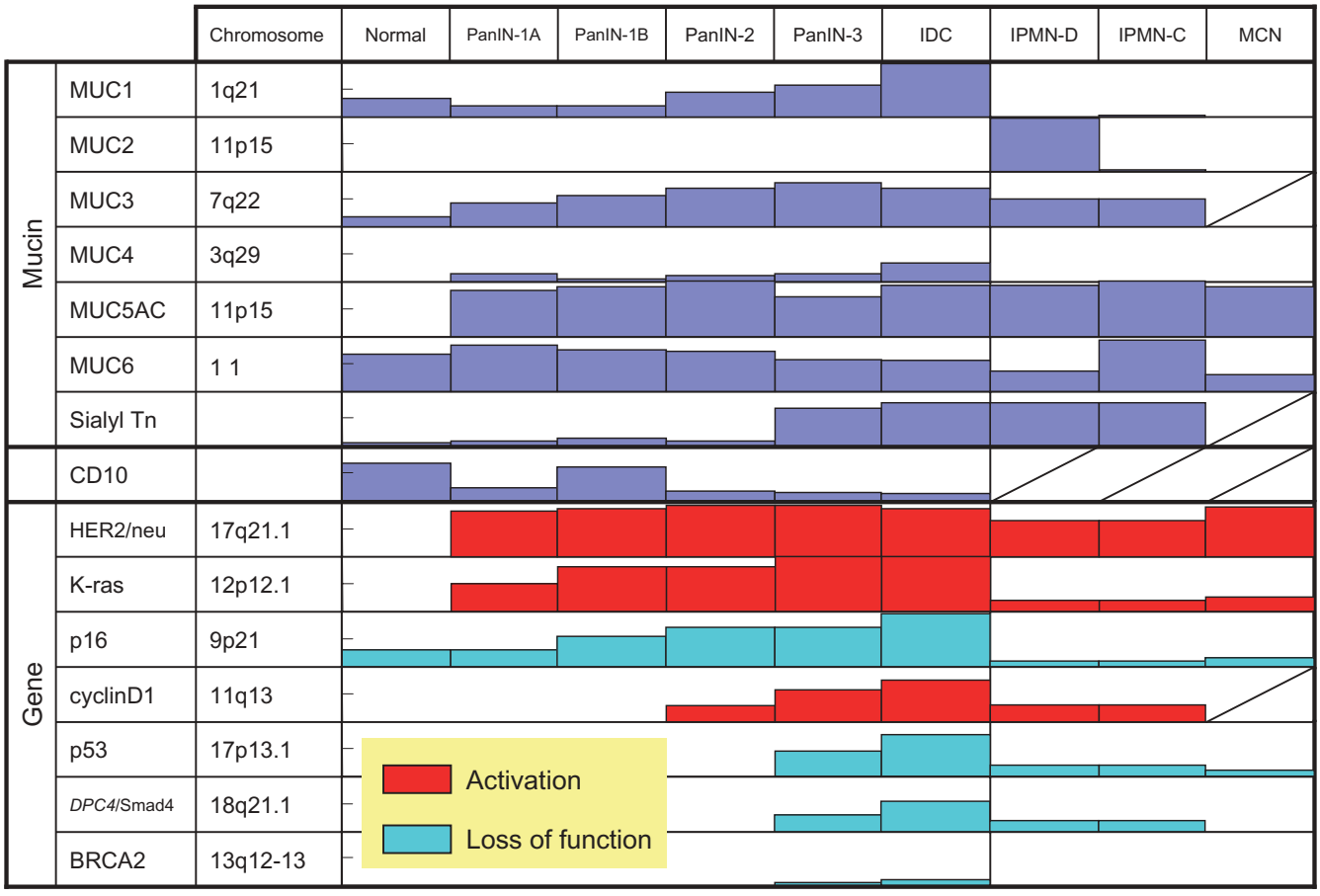

Fig. 5. Summary of the mucin expression profiles and the mutation of various genes in normal duct, pancreatic intraepithelial neoplasia (PanIN), IDC, IPMN, and mucinous cystic neoplasm (MCN). The expression profiles of mucins and CD10 are from our own data.[23,25] Data of sialyl Tn are from reference 28. Gene mutations are from various sources.[29–47] In the data of IPMNs and MCNs, data of the cases carrying the carcinoma are not included

by carbohydrate side chains produced by normal breast epithelial cells, whereas the carbohydrate side chains of MUC1 produced by breast adenocarcinomas are shorter or less densely distributed than those produced by normal cells. However, our recent study disclosed that sialylated or fully glycosylated MUC1 mucins, as well as poorly glycosylated MUC1 mucins, were expressed in breast carcinomas.[22] The expression of various glycoforms of MUC1 mucins was also recognized in the other human carcinomas of the stomach,[16] intrahepatic bile duct,[17] and extrahepatic bile duct.[20] Nakamori et al.[49] also reported that colorectal carcinomas show a high-level expression of fully glycosylated MUC1 mucin in the advanced stages or in the metastatic lesions. Our study was the first report demonstrating that pancreatic IDCs showed high expression of various glycoforms of MUC1; not only poorly glycosylated MUC1 (MUC1/CORE and MUC1/DF3), but also glycosylated MUC1 (MUC1/MY.1E12 and MUC1/HMFG-1). [25]

In our previous study,[21] we reported that most of the IPMNs with MUC2+ were IPMNs-D, whereas most of the IPMNs with MUC2– were IPMNs-C. From the different patterns of MUC2 expression as well as the significant differences in the clinicopathological factors such as incidence of carcinoma and frequency of invasive proliferation between IPMNs-D and IPMNs-C, we believe that IPMNs-D with MUC2+ expression and IPMNs-C with MUC2– expression belong to different lineages of neoplasm, which was confirmed by our next large-scale study. [25]

Our study demonstrated for the first time that there were apparent differences in the expression of glycosylated MUC1 mucins (MUC1/MY.1E12 and MUC1/HMFG-1), in addition to the different expression of MUC2, between IPMNs-D and IPMNs-C.[25] Rare expression of poorly glycosylated MUC1 mucins (MUC1/CORE and MUC1/DF3) was common to the two types of IPMNs, and these data are consistent with the results in our previous studies in which we examined the expression of MUC1/DF3 only.[2,21,50] In contrast, sialylated MUC1 (MUC1/MY.1E12) and fully glycosylated MUC1 (MUC1/HMFG-1) were rarely expressed in IPMNs-D,

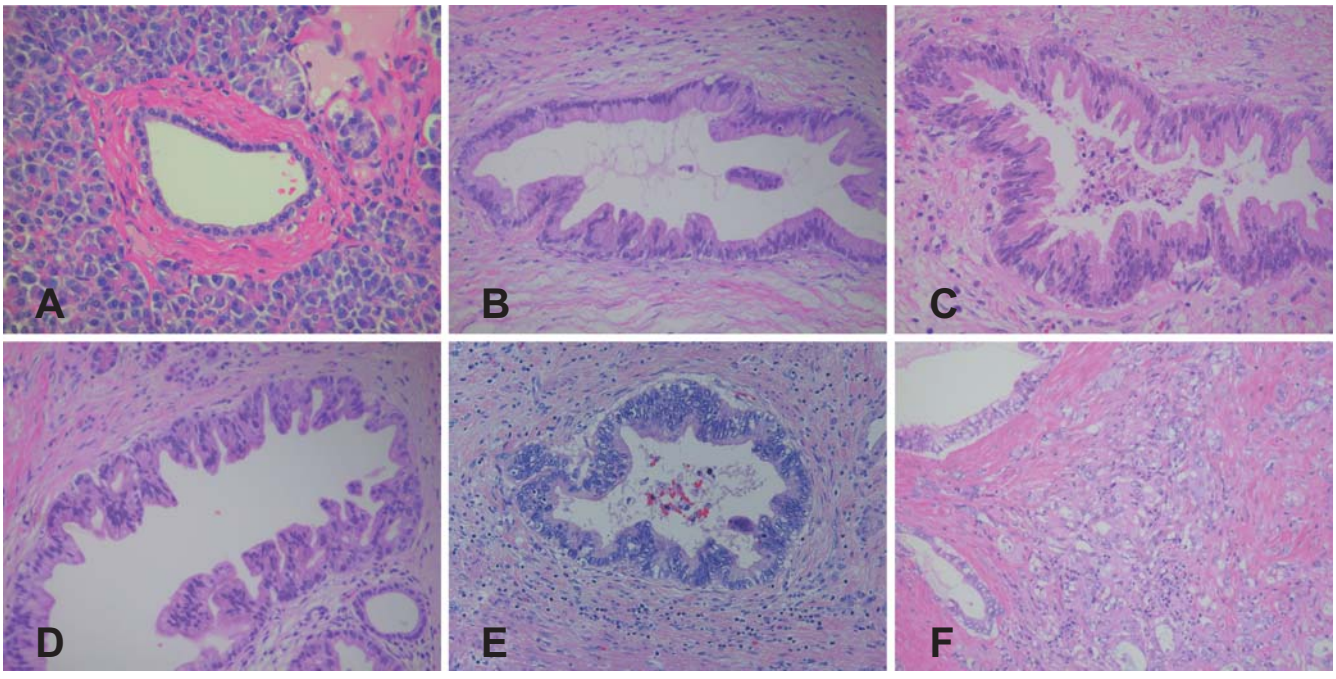

Fig. 6. Histological typing of PanINs. Normal duct (**A**), PanIN-1A (**B**), PanIN-1B (**C**), PanIN-2 (**D**), PanIN-3 (**E**), and IDC (**F**). ×200 (**A**); ×100 (**B**–**F**)

whereas they were expressed in IPMNs-C. The reason why the glycosylated MUC1 mucins (MUC1/MY.1E12 and MUC1/HMFG-1) were expressed only in IPMNs-C was unknown. However, since the expression rates of the glycosylated MUC1 mucins were significantly different between IPMNs-D and IPMNs-C, the different expression pattern of the glycosylated MUC1 mucins between two types of IPMNs may also support the concept that IPMNs-D and IPMNs-C belong to different lineages of neoplasm.

An in situ hybridization study for MUC1 gene expression reported that MUC1 transcript was expressed highly in IDCs,[51,52] but rarely in IPMNs.[52] These findings are consistent with high expression of all the glycoforms of MUC1 mucins in IDCs, and also with rare expression of them in IPMNs-D, as reported in this study. However, expression of MUC1/MY.1E12 and MUC1/HMFG-1 in IPMNs-C shows a discrepancy with the rare expression of MUC1 transcript in IPMNs. An in situ hybridization study for MUC1 in IPMNs-C would be interesting in the future to clarify whether or not the low expression of poorly glycosylated MUC1 (MUC1/CORE and MUC1/DF3) is owing to the masking of the MUC1 core peptide by carbohydrate moieties detected by MAb MY.1E12 or HMFG-1. An in situ hybridization study for MUC1 gene expression based on a clear classification of IPMN is also necessary to clarify the dissociation between in situ hybridization studies and immunohistochemical studies of IPMNs-C.

Adsay et al. classified IPMNs into two types, the intestinal type and the pancreatobiliary type.[53,54] The intestinal type is the same tumor as IPMNs-D (Fig. 3A) in our classification.[21,24,25] On the other hand, we considered that the histological finding of severe atypia or carcinomatous change of IPMNs-C may be the same as the pancreatobiliary type (Fig. 3B),[25] which strongly expressed glycosylated MUC1 (clone Ma695, Vector Laboratories).[54] The "classical IPMN" reported by Terris et al.[52] is the same tumor as IPMNs-D. We are also interested in the relationship between IPMNs-C and "hyperplastic type IPMN" reported by Terris et al.[52]

Expression of MUC2, MUC5AC, and MUC6 mucins (secretory-type mucin)

IDCs showed no expression of MUC2 (0%). IPMNs-D showed a high expression rate of MUC2 (96%), whereas IPMNs-C showed a low expression rate of MUC2 (3%). The expression rate of MUC2 in IPMNs-D was significantly higher than that in IPMNs-C ($P < 0.0001$) or in IDCs ($P < 0.0001$). There was no significant difference in the expression rates of MUC2 between IPMNs-C and IDCs. In summary, MUC2 showed high expression only in IPMNs-D, but no expression or very low expression in IDCs and IPMNs-C.

MUC5AC was expressed in 46/50 IDCs (92%), in 25/27 IPMNs-D (92%), and in all the IPMNs-C 36/36 (100%). In IPMNs, MUC5AC was expressed frequently in the projected areas (Fig. 4W).

MUC6 was expressed in only 28/50 IDCs (56%), whereas it was expressed in 10/27 IPMNs-D (37%) and 33/36 IPMNs-C (92%). The expression rate of MUC6 in IPMNs-C was significantly higher than that in IPMNs-D ($P < 0.0001$) or in IDCs ($P < 0.0005$). In IPMNs, MUC6 was expressed mainly in the basal areas of the papillary lesions, where MUC5AC was negative (Fig. 4X).

MUC2 and MUC5AC are gel-forming mucins which form long polymers by end-to-end disulfate bonding, resulting in molecules with high viscosity in solution.[55–57] Since the production of mucin is a peculiar characteristic of IPMN, we are interested in the expression profiles of the two gel-forming mucins. Our study demonstrated that most of the IPMNs-D showed a MUC2+ and MUC5AC+ expression pattern. The IPMNs-D is similar to colorectal villous adenoma, not only in morphological appearance but also in the expression of both MUC2 and MUC5AC.[58] In contrast, most of the IPMNs-C showed a MUC2– and MUC5AC+ expression pattern. The IPMNs-C is similar to the surface mucous cells of gastric mucosa, not only in morphological appearance but also in the MUC2– and MUC5AC+ expression pattern.[24] The MUC2 and MUC5AC expression patterns in both IPMNs-D and IPMNs-C may be explained in part by their similarity to colorectal villous adenoma and gastric surface mucosa, respectively. However, most IDCs also showed a MUC2– and MUC5AC+ expression pattern, like IPMNs-C. Luttges et al.[37] also reported that MUC5AC showed high positive expression in both IPMNs and IDCs. Both MUC2 and MUC5AC genes are located on the chromosome 11p15.5 region as a cluster of complex mucin genes.[59] The distinct expression pattern between MUC2 and MUC5AC, i.e., MUC2, is peculiar to IPMNs-D only, whereas MUC5AC is common not only to both IPMNs-D and IPMNs-C, but also to IDCs, suggesting differential regulation of these mucin genes. Recent studies have stressed that MUC5AC expression in pancreatic neoplastic lesions is an early event in tumor progression,[28] and that MUC5AC is expressed not only in the neoplastic lesions, but also in the hyperplastic ductal epithelium.[51] Recently, Ho et al.[60] reported a difference in methylation status between the promoter regions of MUC2 and MUC5AC. The relationship between the methylation status of MUC2 and MUC5AC in two types of IPMNs and the expression patterns of MUC2 and MUC5AC in two types of IPMNs is also an interesting future area of study.

MUC6 is also a gel-forming mucin, and is located in the chromosome 11p15.5 region, like MUC2 and MUC5AC.[59] Immunohistochemical studies on the expression of gastric-type secretory mucins revealed that MUC5AC is observed mainly in the surface mucous cells of the cardia, fundus, and antrum of the stomach, whereas MUC6 is observed in the pyloric glands.[61,62] It is an interesting finding that MUC5AC was frequently expressed in the papillary or villous lesions of IPMNs in the projected areas, whereas MUC6 was expressed mainly in the basal areas. This difference of the location of MUC5AC and MUC6 is similar to their location in the stomach. In IPMNs, not only the expression of gastric-type mucins but also the differentiation of their location may be regulated.

The potential for malignancy is also different between the two types of IPMNs.[21,25] Because of the differences in mucin expression patterns and the potential for malignancy between the two types, we believe that it is reasonable to class IPMNs-D and IPMNs-C into different entities. The classification of IPMNs into IPMNs-D and IPMNs-C may be useful for selection of the treatment method, e.g., conservative follow-up or surgical removal of the tumor, and also useful for selection of the surgical method, e.g., radical removal or partial resection of the tumor. A comparison of the two subtypes of IPMNs by using the Kaplan–Meier method indicated that IPMNs-D tended to have a worse prognosis than IPMNs-C. This is mainly owing to the frequent development of invasive mucinous carcinoma in IPMNs-D,[21,26] with MUC1 expression in the invasive lesion of the carcinoma.[21,63] Thus, we must watch patients with IPMN-D extremely carefully.

In conclusion, our studies demonstrated that pancreatic IDCs showed a high expression of all the glycoforms of MUC1, and also that IPMNs can be classified into distinct two types, IPMNs-D and IPMNs-C, which show different expression patterns of glycosylated MUC1, MUC2, and MUC6, and different potentials for malignancy.

Recent study of MUC4 expression in IDCs

In 2004, we reported for the first time that MUC4 was expressed in the carcinoma tissues of 10 (37%) of the 27 intrahepatic cholangiocarcinomas, mass-forming type (ICCs-MF), whereas it was not expressed in normal liver tissue, including bile ducts. The survival rate of patients with MUC4-positive expression was significantly poorer than that of patients with MUC4-negative expression. In addition, the expression of MUC4 in ICCs-MF is a new, independent poor prognostic factor, and is a useful marker to predict the outcome of patients with ICCs-MF.[64]

In the pancreas, MUC4 was not expressed in normal tissue (see Fig. 5). In IDCs, MUC4 was expressed in 43 of 135 patients with IDCs (32%). The survival of 21 patients with high-expression MUC4 (more than 20% of neoplastic cells stained) was significantly poorer than that of 114 patients with low-expression MUC4 (under 20% of the neoplastic cells stained) ($P = 0.0043$). The survival of patients with high-expression MUC4 was

significantly poorer than that of patients with low-expression MUC4 (P = 0.0043, log–rank test). Backward step-wise multivariate analysis showed that MUC4 expression in IDCs is a new independent factor for poor prognosis, and predicts the outcome of patients with IDCs.[65]

MUC4/Sialomucin complex (SMC) is a rat homologue of human mucin gene *MUC4* and its transmembrane subunit acts as an intramembrane ligand for the receptor tyrosine kinase ErbB2 to induce the phosphorylation of Tyr-1248 of the ErbB2.[66–68] MUC4/SMC leads to the expression of the cell-cycle inhibitor p27.[66] On the other side, MUC4/SMC with neuregulin acts synergistically to enhance phosphorylation of both ErbB2 and ErbB3, resulting in the down-regulation of p27 and activation of protein kinase B/Akt.[67,69] It is proposed that the complex formation of MUC4/SMC and ErbB2 has an effect on epithelial cell behaviors, as a switch in epithelial differentiation and proliferation.[67,69] Furthermore, Singh et al.[70] proposed that MUC4 participates in tumor growth and metastasis by directly altering the tumor cell properties, and/or via modulating ErbB2 expression. However, in the IDCs examined in this study, ErbB2 expression was not a significant prognostic factor. The combined evaluation of MUC4 and ErbB2 expression showed no significant result either. Nevertheless, high expression of ErbB2 was more frequently seen in IDCs with good differentiation than in those with poor differentiation. This finding is compatible with the report of colorectal cancers that good or moderately differentiated tumors more frequently expressed ErbB2 proteins than poorly differentiated tumors.[71]

Low expression of p27 is a poor prognostic factor in gastric cancer, colorectal carcinoma, and intrahepatic cholangiocarcinoma.[72–74] In the IDCs examined in this study, however, p27 expression itself was not an independent prognostic factor. In a combined evaluation, patients with high-expression MUC4 and high-expression p27 showed a significantly worse outcome than those with low-expression MUC4 and low-expression p27. This result leads to the suspicion that the p27 up-regulation is induced by MUC4 expression in IDCs. The relationship between the p27 up-regulation and MUC4 expression may be partly explained by the model proposed by Carraway et al.[69] and Jepson et al.[67]

MUC4 extends at least 1.12–2.12 μm above the cell membrane, which is far above all other membrane-associated proteins such as adhesion molecules.[70,75] MUC4, with its rigid and extended structure, is considered to be a modulator for cell–cell and cell–extracellular matrix interactions.[76] Komatsu et al.[76,77] showed that SMC disrupts integrin-mediated cell adhesion to extracellular matrix proteins. In addition, over-expression of SMC masks the surface antigens on target tumor cells and effectively suppresses the killing of tumor cell by cytotoxic lymphocytes.[76] These phenomena may be related to the poor outcome of patients with high-expression MUC4, although MUC4 expression was not related to the morphological invasive parameters such as lymphatic invasion, venous invasion, perineural invasion, and distant lymph node metastasis.[65]

We also confirmed the cytoplasmic expression pattern of MUC4 in IDCs in our study.[65] Like MUC1, MUC4 is a membrane mucin; however, MUC4 acts with different mechanism from MUC1. For signaling molecules, MUC1 acts as a docking protein, whereas MUC4 acts as a receptor ligand.[66] The difference in the expression patterns suggests the possibility of a different mechanism of expression between MUC1 and MUC4, both of which are membrane mucins possessing a cell-signaling function.

In conclusion, important information on the significance of MUC4 expression is a useful indicator for predicting the outcome of patients with IDCs who had surgical resection of the tumor. MUC4 could potentially be a useful marker for the clinical management of patients with IDCs.

Expression profile of mucins in PanINs

Adequate nomenclature for precancerous lesions is essential for effective investigations to understand the carcinogenesis. Nowadays, it is believed that IDCs are developed from histologically well-defined precursor ductal lesions known as PanINs. The PanINs are graded as PanIN-1A, -1B, -2, and -3, according to the histological atypia (see Fig. 6).

In our study, 18 surgically resected lesions of the pancreas (3 specimens with chronic pancreatitis, 10 specimens with intraductal papillary–mucinous adenoma, and 5 specimens with IDCs) were collected.[23] Nine normal ducts, 80 PanINs (PanIN-1A, 35; PanIN-1B, 20; PanIN-2, 18; PanIN-3, 7), and 8 IDCs were selected, and we examined the expression profiles of the mucins in normal ducts; PanIN lesions and IDCs were examined using immunohistochemistry.

The results of our immunohistochemical study of mucin expression in PanINs and IDCs are summarized in Fig. 5.[23] The expression profiles of mucins in all grades of PanINs and IDCs are summarized as follows: (1) over-expression of MUC1 and MUC6, and an increase in MUC1 expression correlated with the grades of PanINs; (2) de novo expression of MUC4 and MUC5AC.

Over-expression of MUC1 in PanINs seems to be a consensus phenomenon among the study groups.[23,28]

Swartz et al. reported that MUC4 expression increases progressively in PanINs, and Park et al. also noted a

progressive increase in MUC4 expression in PanINs.[78,79] They reported that MUC4 was expressed in 89% or 79%, respectively, of IDCs. In contrast, MUC4 was expressed in 32% of IDCs in our recent study,[65] although we used the same antibody and staining method as described by the other two research groups. According to the low expression rate of MUC4 in IDCs in our study, we could not detect a progressive increase of MUC4 expression in PanINs. However, the phenomenon of de novo expression of MUC4 in PanINs shows no discrepancy between our study and the studies by Swartz et al. and Park et al.[79,80]

Kim et al.[28] demonstrated that the expression of MUC5AC and MUC6 in PanIN is an early event. Generally, our results were consistent with their results. However, the expression rate of MUC6 in normal ductal epithelium showed a discrepancy between our study (67%) and their study (25%), although we and they used the same antibody for the immunohistochemical study (clone CLH5, Novocastra).

In conclusion, the expression of both MUC5AC and MUC6 is an early event, whereas up-regulation of MUC1 is a late event in PanINs.

Expression profile of mucins in MCNs

Mucinous cystic neoplasms (MCNs) are rare tumors, and occur almost exclusively in women's body to tail. The lesions show no communication with the pancreatic ductal system. MCNs show multilocular to unilocular epithelial cysts lined by mucin producing cuboidal to columnar cells surrounded by ovarian-like stroma. MCNs are classified as adenoma, borderline (low-grade malignant), noninvasive, or invasive carcinomas by epithelial, structural, and cellular atypia.

Because MCNs are rare, only a few reports are available, and a profile of mucins expression has not yet been established. The expression of MUC1 is mostly absent in the report by Luttges et al.[37] One case in their report showed a positive result, and is suspected to be invasion or undifferentiated carcinoma. On the other hand, Terada et al.[81] reported that MUC1/DF3 was expressed in 7 of 8 cases of mucinous cystadenomas.

Terada et al.[81] did not detect MUC2 in any of their 8 mucinous cystadenomas. Luttges et al.[37] did not find MUC2 expression either except for goblet cells. Zamboni et al.[82] reported that intestinal mucin markers CAR-5 (a marker of colorectal epithelium) and M3SI (a marker of small intestine goblet cells) were expressed more often in mucinous cystadenocarcinomas (38%) than in mucinous cystic adenomas (4%) or mucinous cystic borderline tumors (18%). These results suggest that MCNs acquire intestinal type character with the malignant transformation.

Reports of the expression of MUC5AC vary from 37.5% to 100%.[37,77] The authors used different antibodies, which might explain the different results. MUC5AC is not expressed in normal pancreatic ducts, but were highly expressed in PanINs of all grades, IPMNs, MCNs, and IDCs.[23] Therefore, MUC5AC is de novo expressed from the early phase of tumorigenesis, and the strong expression is maintained up to cancerization. This was not useful for the discrimination of malignancy in mucin producing pancreatic neoplasms.

In the study by Luttges et al.,[37] expression of MUC6 was only seen in a few cells of about 30% cases, and was seen in a few acinar cells but not in ductal cells. They reported that MUC6 was not expressed in normal ductal epithelium. On the other hand, we observed MUC6 expression in normal ductal epithelium in 67% of the cases examined, although they and we used the same antibody (clone CLH5, Novocastra).

In conclusion, according to the literature review, MCNs do not seem to show any specific expression profile of mucins.

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