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Fascin overexpression in intraductal papillary mucinous neoplasms (adenomas, borderline neoplasms, and carcinomas) of the pancreas, correlated with increased histological grade

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Intraductal papillary mucinous neoplasm (IPMN) is a well-established entity in pancreatic neoplasms and a precursor of infiltrating adenocarcinoma. Fascin, an actin-bundling protein involved in cellular motility, is upregulated in many human neoplasms. Its overexpression in pancreatic intraepithelial neoplasia, a precancerous lesion sharing many characteristics with IPMN, has been reported. However, fascin expression in IPMN remains unknown. The aim of this study was to investigate fascin expression in IPMNs and to elucidate its relationship to clinicopathological features, including histological grade and phenotypic subclassification. We evaluated fascin expression by immunohistochemistry in 116 surgical specimens, followed by quantitative analysis of fascin mRNA expression using a laser microdissection system and real-time reverse-transcriptase polymerase chain reaction in eight frozen samples. Fascin expression was significantly higher in borderline neoplasms (25/29, 86%) and carcinomas (37/42, 88%) than in adenomas (23/45, 51%) ($P<0.05$, respectively), but no difference was observed between borderline neoplasms and carcinomas. With regard to the subclassification, intestinal-type neoplasms (35/39, 90%) were more frequently positive for fascin than gastric-type neoplasms (36/59, 61%) ($P<0.05$). Two oncocytic-type neoplasms were both fascin-negative. Fascin mRNA expression seemed to be higher in moderately to severely dysplastic epithelium than in mildly dysplastic epithelium (not statistically significant), supporting the immunohistochemical experiments. Our findings suggest that fascin overexpression is involved in the progression of IPMN. Fascin could become a new therapeutic target for inhibition of their progression.
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Intraductal papillary mucinous neoplasm (IPMN) is a well-established entity in pancreatic neoplasms. It was first reported in 1982 as a special type of pancreatic neoplasm with a characteristic endoscopic finding of extrusion of mucin through the ampulla of Vater.[1] At present, the term is used to unify tumors characterized by intraductal proliferation of neoplastic mucinous epithelium, which usually forms papillae and leads to cystic dilation of the pancreatic ducts.[2,3] Because IPMNs show a broad spectrum of dysplasia ranging from adenoma and borderline neoplasm to carcinoma *in situ*, the existence of an adenoma–carcinoma sequence is probable. In addition, some IPMNs are associated with infiltrating adenocarcinoma. Therefore, IPMN is gaining attention as a precursor of infiltrating adenocarcinoma in the pancreas as well as pancreatic intraepithelial neoplasia (PanIN).[4–6] Investigation of factors correlated with the progression of noninvasive and/or invasive IPMNs is important.

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Several authors have proposed subclassification systems for IPMN based on histological phenotypes and/or immunohistochemical profiles of mucin core protein (MUC) expression.[7–13] Recently, a consensus on the subclassification of IPMNs was agreed among international experts on pancreatic precursor lesions, and published.[14] Subclassification makes it easier to compare studies among different institutions and understand the biological behavior, and is essential for future studies of IPMN.

Fascin-1 (also known as fascin) is a globular actin cross-linking protein. It is required for the formation of actin-based cell-surface protrusions that are essential for cellular migration and cell–matrix adhesion.[15–17] In normal epithelial cells, fascin expression is usually absent or very low, but it is significantly upregulated in transformed epithelial cells and several types of human carcinoma such as lung,[18,19] breast,[20–23] esophagus,[24,25] stomach,[26] colon,[27] pancreas,[28–31] biliary tract and ampulla,[31,32] ovary,[33] urinary bladder,[34] and skin.[35] Among the above neoplasms, fascin upregulation is most frequently observed in pancreatic infiltrating adenocarcinoma.[28,30,31] It is interesting that PanIN shows fascin expression despite being an intraepithelial neoplasia.[29,30] In general, in tumors of other organs, expression of fascin is especially strong in areas of infiltration, or is limited to such areas. Fascin expression in IPMN and its relationship with the clinicopathological features remain unclear, though IPMN has much in common with PanIN.

The aims of the present study were to analyze fascin expression using a large number of surgical IPMN specimens, to clarify its relationship with clinicopathological features including histological grade and phenotypic subtype, and to elucidate the association of fascin expression with progression of IPMNs.

Materials and methods

Patients and Tissue Specimens

A total of 116 samples of IPMN were used for the present study. All of the neoplasms were surgically resected at Kyushu University Hospital and its affiliated hospitals from 1986 to 2005. All specimens were cut into 5 mm stepwise tissue sections, and the gross features were recorded. For histopathological diagnosis, they were embedded in paraffin, and each of the serially cut sections, 4 μm in thickness, was stained with hematoxylin and eosin (H&E). On the basis of the greatest degree of dysplasia present, the lesions were classified as adenoma, borderline neoplasm, or carcinoma with or without invasion according to the World Health Organization (WHO) classification.[2] If present, invasive components were classified as tubular or mucinous noncystic (colloid) type. In accordance with the recently suggested subclassification system,[14] the lesions were also subclassified into four groups, gastric type, intestinal type, pancreatobiliary type, and oncocytic type, based on their histological phenotype and immunohistochemical expression of MUCs; gastric type MUC5AC+/MUC2−/MUC1−, intestinal type MUC5AC+/MUC2+/MUC1−, pancreatobiliary type MUC5AC+/MUC2−/MUC1+, and oncocytic type MUC5AC+/MUC2−/MUC1+. The subclassification was based primarily on histological phenotype and the immunolabeling for MUCs served as a confirmatory marker. IPMNs that could not be categorized specifically into one of the above four subtypes were segregated as unclassified type.

We also examined 10 cases of conventional pancreatic ductal adenocarcinoma for fascin expression, immunohistochemically. In addition, eight fresh-frozen samples of IPMN were obtained for quantitative analysis of fascin mRNA.

Immunohistochemistry

The primary antibodies used were follows as: anti-fascin (monoclonal, 55K-2; DAKO, CA, USA; 1:50 dilution), anti-MUC1 (Ma695; Novocastra Laboratories, Newcastle upon Tyne, UK; 1:200 dilution), anti-MUC2 (Ccp58; Novocastra Laboratories; 1:200 dilution), and anti-MUC5AC (CLH2; Novocastra Laboratories; 1:200 dilution). Sections were cut at 4 μm thickness from paraffin-embedded material, then dewaxed with xylene and rehydrated through a graded series of ethanol. After inhibition of endogenous peroxidase and antigen retrieval (microwave irradiation in citrate buffer for all the antibodies), sections were exposed to each primary antibody at 4°C overnight, and stained with a streptavidin–biotin–peroxidase kit (Nichirei, Tokyo, Japan). The sections were then finally reacted in 3,3′-diaminobenzidine, counterstained with hematoxylin, and mounted.

Evaluation of Immunohistochemical Staining of Fascin

Because IPMNs often show variability of epithelial dysplasia within the same tumor, immunohistochemical staining for fascin was evaluated within the area showing the highest degree of dysplasia in each neoplasm. The proportion of fascin positivity was measured using the following scale according to the percentage of fascin-positive tumor cells: $<10\%$, 0; 10–30%, 1+; 30–60%, 2+; and $>60\%$, 3+. Score 0 tumors were considered fascin-negative, whereas the others (1+ to 3+) were considered positive. All slides were evaluated independently by two investigators (HY and TI) without any prior knowledge of the patients' clinical information.

Microdissection and Extraction of Total RNA

We obtained total RNA from individual frozen samples using a laser microdissection system as

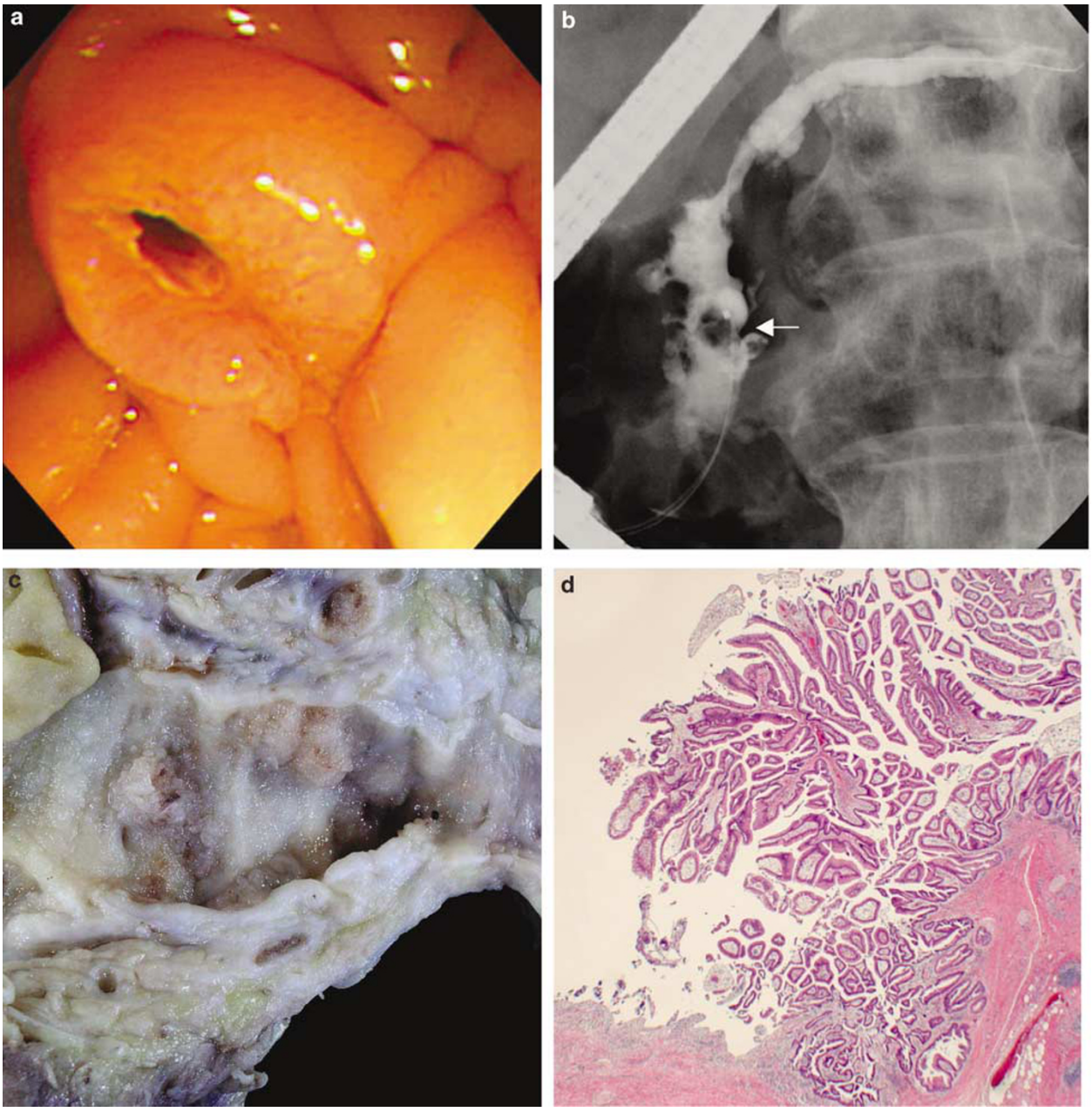

Figure 1 A representative case (75 years old, male) with typical findings of IPMN. (**a**) Endoscopic findings. Characteristic mucin extrusion through the ampulla of Vater. (**b**) Radiographic findings. Dilated main pancreatic duct (MPD) with mural nodule detected as a filling defect (arrow). (**c**) Macroscopic findings (MPD was opened at ventral side). Velvet-like appearance of the surface of a mural nodule. (**d**) Microscopic findings. Intraductal proliferation of neoplastic mucinous epithelium forming a papillary projection. Histological diagnosis is carcinoma.

described previously.[36] In brief, frozen tissue samples embedded in optimum cutting temperature compound (Sakura, Tokyo, Japan) were cut into 8-μm-thick sections. One section was stained with H&E for histological examination. IPMN cells were isolated selectively using laser microdissection and a pressure catapulting system (LMPC; Palm Microlaser Technologies AG, Bernried, Germany) in accordance with the manufacturer's protocols. After microdissection, total RNA was extracted from the selected cells according to the standard acid guanidinium thiocyanate–phenol–chloroform protocol[37] with glycogen (Funakoshi, Tokyo, Japan), and was subjected to real-time reverse-transcriptase polymerase chain reaction (RT-PCR) for quantitative measurement of fascin mRNA.

Quantitative Analysis of Fascin mRNA Expression by Real-Time RT-PCR

We designed a real-time RT-PCR protocol for the quantitative analysis of fascin mRNA and a refe-

rence gene, 18S ribosomal RNA (18S rRNA). We designed specific primers (fascin forward primer, 5′-gcaccctcaggtcaacatct-3′; reverse primer, 5′-aactccagc gtgtagccagt-3′; 18S rRNA forward primer, 5′-gatatgct catgtggtgttg-3′; reverse primer, 5′-aatcttcttcagtcgctc ca-3′), and used Basic Local Alignment Search Tool analysis to ensure the gene specificity of these primers. Quantitative one-step RT-PCR was carried out with a Quantitect SYBR Green RT-PCR kit (QIAGEN, Tokyo, Japan), and a LightCycler Quick System 350S (Roche Diagnostics, Mannheim, Germany), according to the manufacturers' instructions. In brief, the total volume of the reaction mixture was 20 μl, containing 10 μl of 2 × SYBR Green Buffer, 0.2 μl of RT mix, 1 μl of each primer (10 μmol/l), and 1 μl of total RNA. The reaction mixture was first incubated at 50°C for 15 min to allow reverse transcription. PCR was then initiated at 95°C for 10 min to activate modified Taq polymerase, followed by a 45-cycle amplification (95°C for 15 s, 55°C for 20 s, and 72°C for 10 s) and one cycle (95°C for 0 s, 65°C for 15 s, and 0.1°C/s to 99°C) for melting analysis. Each sample was run in triplicate. The mRNA expression of each gene was calculated on a standard curve constructed using total RNA from the MRC5 fibroblast cell line. For relative quantification, expression of fascin mRNA was normalized to that of 18S rRNA.

Table 1 Clinicopathologic findings of resected intraductal papillary mucinous neoplasms (IPMNs)

Parameters	*Number*
Age (years, mean ± s.d.)	66.34 ± 8.15
Sex	
Male	76 (66%)
Female	40 (34%)
Site	
Head	76 (66%)
Body and/or tail	39 (34%)
Tumor size (mm) (median (25, 75%))	30 (20, 40)
Mural nodule	
Absent	71 (61%)
Present	44 (38%)
Histological grade (WHO classification)	
Adenoma	45 (39%)
Borderline neoplasm	29 (25%)
Carcinoma	42 (36%)
Noninvasive	20 (17%)
Invasive	22 (19%)

s.d., standard deviation; WHO, World Health Organization.

Statistical Analysis

The χ^2 test was used to evaluate the association between histological grade and fascin expression. Fisher's exact test was used to assess the association between subtype and fascin expression or histological grade. After these analyses, multiple comparisons were carried out using Bonferroni's method. Spearman rank correlation analysis was used to study the relationship between histological grade and fascin score (0–3+). In the other analyses, the χ^2 test or Fisher's exact test was used for proportion, and the *t*-test, Mann–Whitney's *U*-test, analysis of variance, or the Kruskal–Wallis test was used for continuous data. *P*-values of less than 0.05 were considered statistically significant.

Results

Clinicopathological Features and Phenotypic Subclassification

A representative case showing typical endoscopic, radiographic, macroscopic and microscopic findings is shown in Figure 1. The clinicopathological findings from 116 cases are summarized in Table 1. Among the parameters, increasing tumor size ($P<0.0001$) and a presence of a mural nodule ($P<0.0001$) were significantly correlated with increased histological grade (adenoma–borderline neoplasm–carcinoma) (data not shown).

Of the 116 lesions, 59 (51%) were subclassified as gastric type, 39 (34%) as intestinal type, seven (6%)

Table 2 Subtype and histological grade (WHO classification)

	Adenoma	*Borderline*	*Carcinoma*			P
	n *(%)*	n *(%)*	n *(%)*	*Noninvasive*	*Invasive (tub:muc)*	
Sub-type						<0.0001
G-type ($n=59$)	41 (69)	13 (22)	5 (8)	1	4 (4:0)	
I-type* ($n=39$)	4 (10)	16 (41)	19 (49)	12	7 (3:4)	
PB-type* ($n=7$)	0 (0)	0 (0)	7 (100)	1	6 (6:0)	
O-type ($n=2$)	0 (0)	0 (0)	2 (100)	1	1 (1:0)	
U-type* ($n=9$)	0 (0)	0 (0)	9 (100)	5	4 (3:1)	

G-type, gastric type; I-type, intestinal type; muc, mucinous noncystic (colloid) invasive pattern; O-type, oncocytic type; PB-type, pancreatobiliary type; Tub, tubular invasive pattern; U-type, unclassified type.
*$P<0.05$ significantly increased histological grade compared with G-type, using Bonferroni's method.

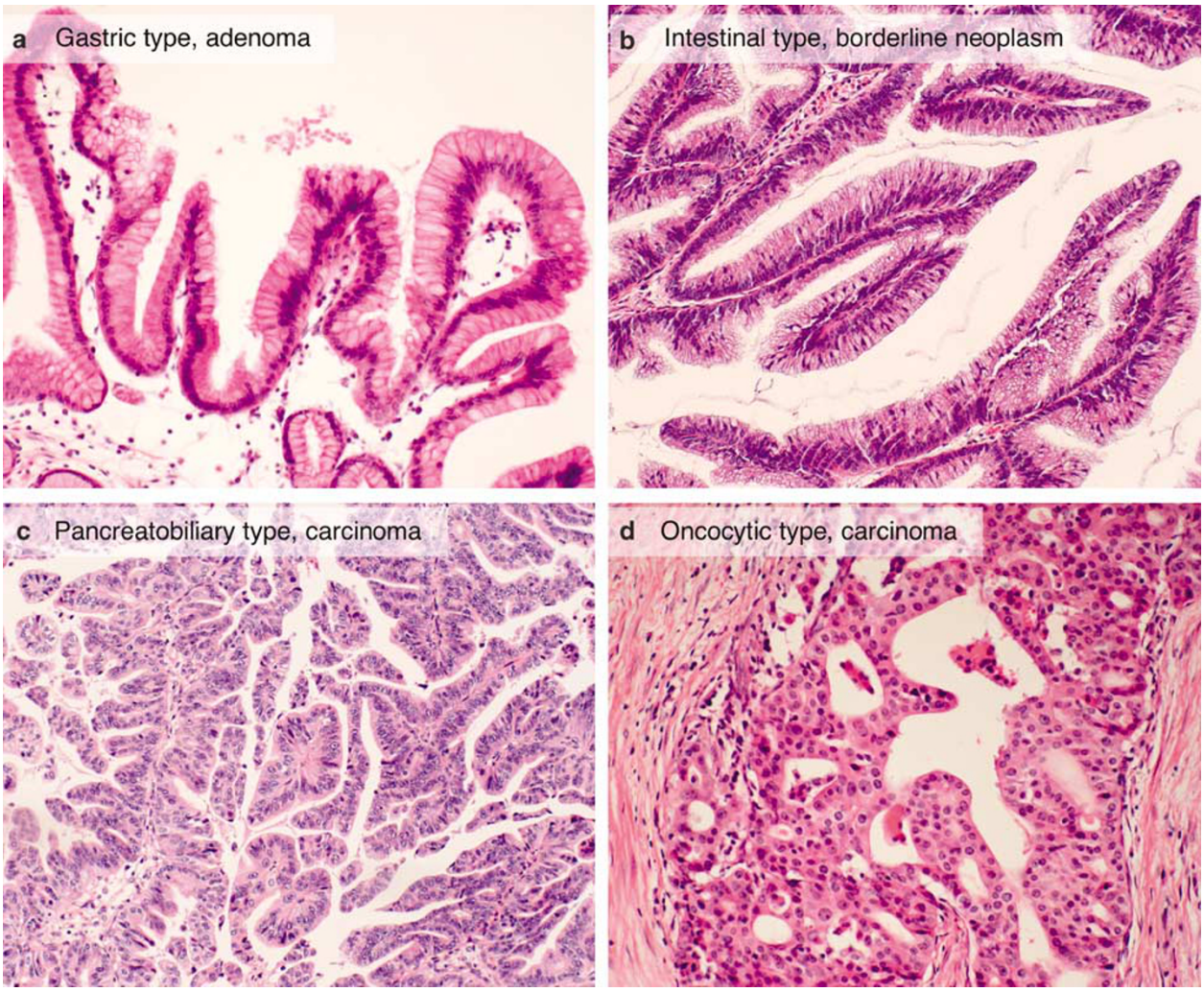

Figure 2 Representative images of the subtypes of IPMN stained with H&E. (**a**) Gastric-type IPMN consisting of cells resembling gastric foveolae with mild epithelial dysplasia. (**b**) Intestinal-type IPMN resembling intestinal villous neoplasms showing tall, columnar epithelial cells with moderate epithelial dysplasia. (**c**) Pancreatobiliary-type IPMN consisting of cells resembling cholangiopapillary neoplasms showing complex, thin, branching papillae with severe epithelial dysplasia. (**d**) Oncocytic-type IPMN consisting of cells with abundant, intensely eosinophilic cytoplasm and showing complex papillae with intraepithelial lumina and severe epithelial dysplasia.

as pancreatobiliary type, two (2%) as oncocytic type, and nine (8%) as unclassified type (Table 2). Each had characteristic papillae formation (Figure 2) and specific immunohistochemical reactivities for MUCs, as described previously.[14] All of the invasive components in the gastric-, pancreatobiliary-, and oncocytic-type neoplasms (four, six, and one neoplasms, respectively) displayed a tubular invasive pattern, whereas four of seven (57%) intestinal-type neoplasms and one of four (25%) unclassified-type neoplasms with invasive components showed a mucinous noncystic (colloid) invasive pattern. By multiple comparisons, intestinal-, pancreatobiliary-, and unclassified-type neoplasms showed significantly increased histological grade compared with gastric-type neoplasm ($P<0.05$; Table 2). Gastric-type neoplasms were significantly smaller (median diameter, 30 mm) than those of intestinal type (30 mm; $P<0.05$, data not shown).

Immunohistochemical Expression of Fascin

Of 116 IPMNs, 85 (73%) demonstrated positive immunohistochemical expression of fascin (Table 3). The immunohistochemical expression appeared as fine granular to diffuse cytoplasmic staining. In every slide prepared for immunohistochemistry, endothelial cells, lymphocytes, and stromal fibroblasts showed positive expression, and these were considered to be internal positive controls. Normal pancreatic ductal epithelium, acini, and islets of Langerhans were essentially nonreactive (Figure 3a); however, some parts of the hyperplastic ductal epithelium surrounding the IPMNs occasionally showed weak positivity for fascin. In addition, squamous metaplasia of the ductal epithelium was also weakly stained in one case. Compared with adenomas (23/45, 51%), the number of fascin-positive neoplasms was significantly higher among borderline neoplasms (25/29, 86%;

Table 3 Correlation between fascin expression and histological grade (WHO classification) or phenotypic subtype

Factor	*Fascin expression*		P
	−n *(%)*	+ n *(%)*	
Histological grade			<0.0001
Adenoma	22 (49)	23 (51)	
Borderline*	4 (14)	25 (86)	
Carcinoma*	5 (12)	37 (88)	
Phenotypic subtype			0.0005
G-type	23 (39)	36 (61)	
I-type**	4 (10)	35 (90)	
PB-type	2 (29)	5 (71)	
O-type	2 (100)	0 (0)	
U-type	0 (0)	9 (100)	
Total	31 (27)	85 (73)	

G-type, gastric type; I-type, intestinal type; O-type, oncocytic type; PB-type, pancreatobiliary type; U-type, unclassified type.
*$P<0.05$ compared with adenoma, using Bonferroni's method.
**$P<0.05$ compared with gastric type, using Bonferroni's method.

$P<0.05$) and carcinomas (37/42, 88%; $P<0.05$) (Table 3, Figure 3b–d). No difference was observed between borderline neoplasms and carcinomas. The fascin score was significantly raised in relation to increased histological grade (Figure 4; $P<0.001$). Within each neoplasm, high-grade-areas often showed more diffuse and intense immunoreactivity for fascin than low grade-areas (Figure 3e). All invasive components seen in the slides prepared for immunohistochemical staining were positive for fascin in both tubular and mucinous noncystic (colloid) patterns (Figure 3f). Intestinal-type neoplasms were more frequently positive for fascin (35/39, 90%) than gastric-type neoplasms (36/59, 61%) ($P<0.05$; Table 3). Two oncocytic-type neoplasms were both negative for fascin. Several parameters (age, sex, tumor site, tumor size, and absence or presence of mural nodule) had no correlation with fascin expression (data not shown).

Of the conventional pancreatic ductal adenocarcinomas, 90% (9 of 10) showed diffuse and intense fascin positivity (Figure 3h), the rate being as high as previously reported.[30,31]

Expression of Fascin mRNA

To confirm the fascin overexpression demonstrated by immunohistochemistry and to assess whether the overexpression was transcriptional or post-transcriptional, real-time RT-PCR for quantitative evaluation of fascin mRNA was performed, using frozen samples of IPMNs. A laser microdissection system was used to isolate IPMN cells for the following purposes: (1) to collect selectively the neoplastic cells showing the same degree of dysplasia within a neoplasm; and (2) to avoid contamination with stromal tissue, which contains many endothelial cells and/or fibroblasts that normally express fascin. The eight frozen samples comprised four samples of mild epithelial dysplasia (corresponding to adenoma) and four of moderate to severe epithelial dysplasia (borderline neoplasm to carcinoma).

The relative expression of fascin mRNA of each sample evaluated by real-time PCR is shown in Figure 5. A tendency was recognized whereby fascin mRNA levels in moderately to severely dysplastic epithelium were higher than those in mildly dysplastic epithelium, though the difference was not statistically significant, possibly because of the small number of samples. These data were consistent with the results of immunohistochemistry, and suggested that the overexpression of fascin in IPMNs was transcriptional.

Discussion

In the present study, we found that fascin was upregulated in the majority (73%) of IPMNs and was correlated with increased histological grade. These findings suggest that fascin overexpression is involved in the progression of IPMN. To our knowledge, this is the first report to reveal fascin overexpression and its association with clinicopathological features in IPMNs, and to evaluate quantitatively the expression of fascin mRNA in human neoplasms using laser microdissection and real time RT-PCR.

Fascin is a well-conserved actin-regulatory protein. *In vitro* experiments have indicated that it is involved in cellular processes such as motility,[27,38,39] loss of cell–cell contact in relation to adhesion molecules,[39–41] and cell proliferation.[25,27] From these observations, it may be presumed that fascin may play an important role in cellular malignant transformation. Indeed, in a variety of human carcinomas, fascin expression is consistently associated with the clinical aggressiveness of the tumor.[18,19,21,23,24,26,33]

Among the pancreatic neoplasms, Iacobuzio-Donahue *et al*[42] reported fascin upregulation for the first time in ductal adenocarcinoma using cDNA microarrays (13-fold overexpression of fascin transcripts in ductal adenocarcinoma compared with normal tissues). Immunohistochemical studies then confirmed fascin overexpression, not only in infiltrating ductal adenocarcinomas[28,30,31] but also in PanINs.[29,30] The fact that PanINs show fascin upregulation correlated with histological grade increased our interest in fascin expression in IPMNs, because PanINs and IPMNs share the following fundamental characteristics:[5,6] inherently intraductal; composed predominantly of columnar, mucin-producing cells that may grow in a flat configuration or may produce papillae; exhibit a range of cytologic and architectural atypia (mild, moderate and severe); recognized as precursors to

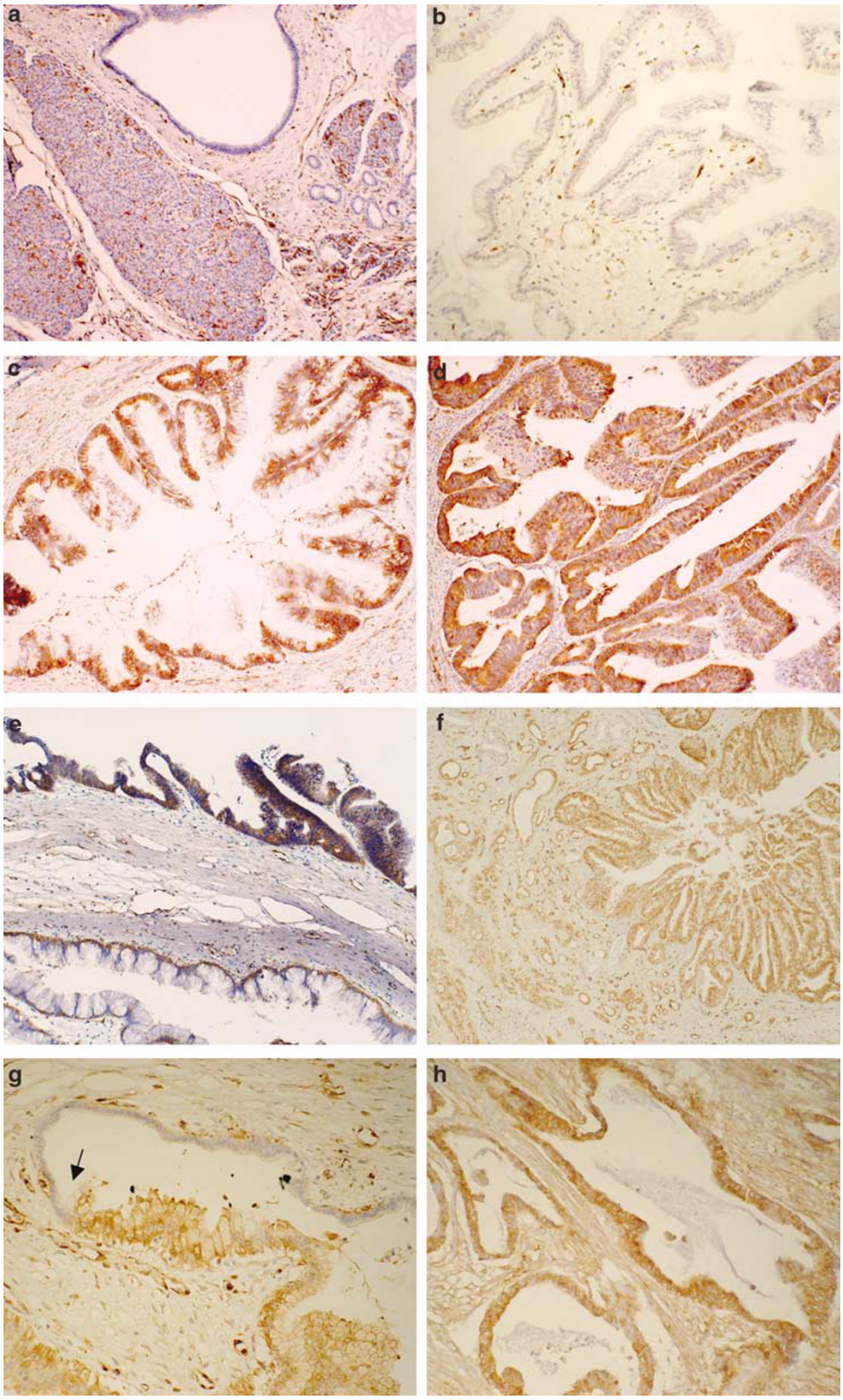

Figure 3 Fascin expression on immunohistochemistry. (**a**) Negative for normal pancreatic ductal epithelium, acini, and islets of Langerhans, whereas positive for endothelial cells and stromal fibroblasts (internal control). (**b**) Negative case of adenoma (score 0). (**c**) Positive case of borderline neoplasm (score 3+). (**d**) Positive case of carcinoma (score 3+). (**e**) Diffuse and intense reactivity in moderately to severely dysplastic epithelium (upper), with weak and basally localized reactivity in mildly dysplastic epithelium (lower) within a neoplasm. (**f**) Positive for both intraepithelial components (right upper) and invasive components (left lower). (**g**) Abrupt transition (arrow) from fascin-positive neoplastic epithelium to fascin-negative normal duct epithelium. (**h**) Diffuse and intense positivity in conventional pancreatic ductal adenocarcinoma.

invasive adenocarcinoma; and sequentially accumulate similar genetic alterations with increasing cytoarchitectural atypia.[43–45]

We showed that fascin overexpression in IPMNs was correlated with increased histological grade by immunohistochemical analysis, followed by a supporting molecular experiment that showed upregulation of fascin mRNA. We consider that fascin upregulation would be a relatively early event in the progression of IPMN, because of the finding that fascin expression was significantly and almost equally greater in borderline neoplasms (86%) and carcinomas (88%) than in adenomas (51%). It would be interesting to compare these results with findings for PanINs. In an immunohistochemical study, Maitra *et al*[30] detected focal or diffuse cytoplasmic fascin expression in 25% of PanIN-1A, 28% of PanIN-1B, 57% of PanIN-2, and 57% of PanIN-3,

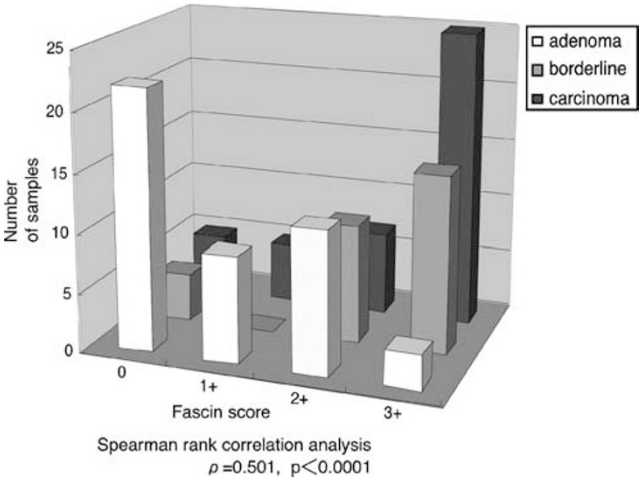

Figure 4 Correlation between histological grade and fascin score. Fascin score was significantly raised in relation to increased histological grade.

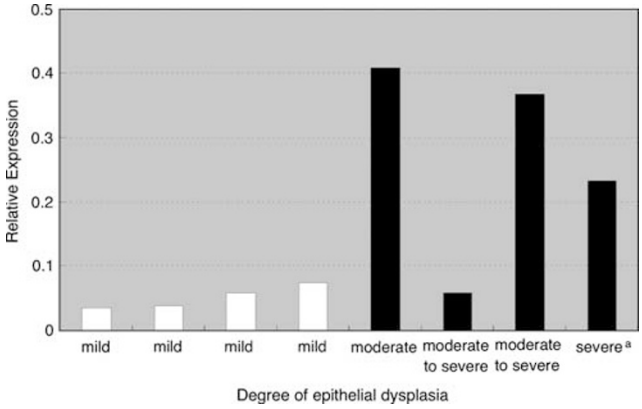

Figure 5 Relative expression of fascin mRNA evaluated by real-time RT-PCR. The relative expression of fascin in eight frozen samples was normalized to18s rRNA. Higher in moderately to severely dysplastic epithelium (black) than in mildly dysplastic epithelium (white). [a]Severe dysplasia with a little invasive components.

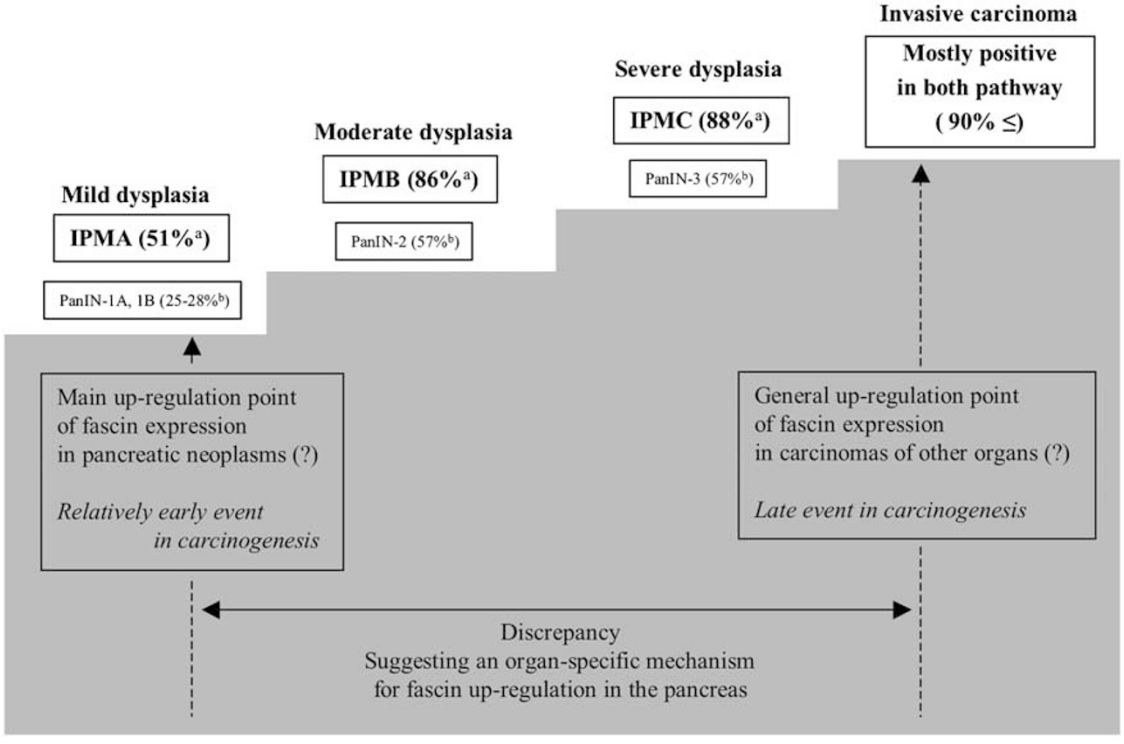

Figure 6 Hypothesis for fascin upregulation in multi-step carcinogenesis of pancreatic neoplasms. IPMA, intraductal papillary mucinous adenoma; IPMB, borderline IPMN; IPMC, intraductal papillary mucinous carcinoma. [a]Percentage of fascin-positive IPMNs documented in the present study. [b]Percentage of fascin-positive pancreatic intraepithelial neoplasms documented in Maitra *et al*.[30]

and described fascin upregulation as an 'intermediate' event in pancreatic adenocarcinoma progression. Their results and ours suggest that upregulation of fascin occurs in similar stage (relatively early phase) in tumorigenesis of both IPMNs and PanINs (Figure 6). It remains to be seen whether fascin expression represents merely a surrogate marker of histological grade, or whether it plays a pathogenic role in tumorigenesis and the progression of IPMNs. Using RNA interference, it has been shown recently that down-regulation of fascin has inhibitory effects on the migration, proliferation and invasiveness of esophageal squamous cell carcinoma cell lines.[24,25] These data suggest that fascin itself contributes to tumor progression, and raise the possibility that fascin could be a novel therapeutic target.

A previous report suggested that a pathway for fascin upregulation was dependent on amplification or overexpression of c-erbB-2/HER-2.[20] Others have shown a possible influence of Wnt signaling on fascin activity, suggesting that anomalies of this pathway may upregulate fascin expression in cancer cells.[40] However, the mechanism of fascin upregulation in IPMN is not known, because neither c-erbB-2 amplification nor Wnt signaling abnormalities are particularly common in IPMN. We consider that there is an organ-specific mechanism for fascin upregulation in the pancreas, because invasive pancreatic adenocarcinomas express prominently high levels of fascin compared with other carcinomas. In addition, IPMNs and PanINs frequently show fascin expression though they are both intraepithelial lesions. Fascin upregulation is not frequently recognized in intraepithelial neoplasms of other organs (Figure 6).

We also performed phenotypic subclassification of IPMNs, and re-confirmed the findings described previously. Gastric types usually showed mild dysplasia and intestinal types showed moderate to severe dysplasia, whereas pancreatobiliary and oncocytic types showed severe dysplasia corresponding to carcinoma *in situ*;[8,10,14] intestinal-type neoplasms were more frequently associated with a mucinous noncystic (colloid) invasive pattern compared with other types;[4,7–9,12] and the oncocytic type was a rare variant.[46] With regard to the relationship between fascin expression and the phenotypic subclassification, fascin overexpression occurred more frequently in intestinal-type neoplasms (90%) than in the gastric type (61%) by multiple comparisons. In contrast, intestinal-type neoplasms exhibited a higher histological grade than the gastric type. It remains unclear whether the difference in fascin overexpression in gastric- and intestinal-type neoplasms is affected by their histological grade, or whether there is a more essential mechanical association between fascin overexpression and subtype. For example, MUC variation may have some molecular relation to fascin expression that has not been documented. Interestingly, two oncocytic-type neoplasms in the present study were both fascin-negative, though they showed severely dysplastic epithelium corresponding to carcinoma. This may indicate that the progression pathway of oncocytic-type neoplasms differs from that of the other types in parts.

In conclusion, overexpression of fascin is correlated with increased histological grade of IPMN and occurs relatively early in the pathogenesis of IPMN. Fascin may provide a new cancer prevention strategy as a possible therapeutic molecular target to inhibit the progression of IPMNs.

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