Immunohistochemical Antibody Panel for the Differential Diagnosis of Pancreatic Ductal Carcinoma From Gastrointestinal Contamination and Benign Pancreatic Duct Epithelium in Endoscopic Ultrasound-Guided Fine-Needle Aspiration

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Objectives: The diagnosis of pancreatic ductal adenocarcinoma (PDAC) by endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) can be challenging to distinguish tumor cells from benign epithelium (BE). The aim of the present study was to set a minimal antibody panel to differentiate PDAC from contaminated BE in EUS-FNA specimens.

Methods: Immunohistochemistry using claudin 4, EZH2, Ki-67, maspin, p53, and S100P was performed on tissue microarray sections containing 53 PDACs and 33 BE as well as cell blocks of EUS-FNA including 53 PDACs and 22 BE. The positive rate was scored as 0 to 4+. The receiver operating characteristic curve was applied to determine a cutoff point, and the Classification And Regression Trees method was used to obtain a classification tree of the best panel.

Results: The cutoff point was 1+ for claudin 4, EZH2, Ki-67, p53, and S100P and 2+ for maspin. All BE scored 0 for p53. The classification tree revealed using p53, S100P, and claudin 4 was the most powerful. The sensitivity and specificity of the tree were 96.2% and 100% in tissue microarrays and 100% and 95.5% in EUS-FNA, respectively.

Conclusions: The classification tree using p53, S100P, and claudin 4 seems to successfully distinguish PDAC from the accompanying BE.

Key Words: endoscopic ultrasound-guided fine-needle aspiration, tissue microarray, immunohistochemical panel, cell block, pancreatic ductal adenocarcinoma

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Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) for pancreatic lesions is a safe and efficient procedure and has become a popular approach for the diagnosis of pancreatic tumors.[1,2] The sensitivity of a cytological diagnosis in EUS-FNA (91.5%) for pancreatic ductal adenocarcinoma (PDAC) was previously reported to be higher than that of pancreatic juice (21.3%) and brushing cytology (48%),[1,3] and the number of cases undergoing EUS-FNA has been increasing in our institute. Recent studies reported the potential of neoadjuvant therapy to decrease the size of tumors and make them more resectable[4,5]; therefore, a definite diagnosis of PDAC in EUS-FNA is important for all patients with PDAC. The EUS-FNA has the ability to accurately diagnose high-grade PDAC; however, well-differentiated PDAC can be challenging because it shows low to moderate N/C ratio, honeycomb structure with mild loss of polarity, low-grade nuclear atypia, and is difficult to distinguish from contaminated benign gastrointestinal epithelium or benign or reactive pancreatic ductal cells, which are present with pancreatitis.[6]

In addition to a cytological diagnosis, the usefulness of cell blocks in EUS-FNA using immunohistochemical staining with various antibodies has recently been reported.[1,6–9] Potential markers for PDAC include enhancer of zeste homologue 2 (EZH2), claudin 4, S100P, Ki-67, and p53.[1,6–17]

The EZH2 is a methyltransferase at lysine 27 of histone H3 and is also a member of the developmentally related polycomb repressor complex.[10] An increase in the expression of EZH2 has been detected in a number of carcinomas including PDAC and cholangiolocellular carcinoma.[10–12]

Claudin 4 is a protein involved in the formation of tight junctions. Its overexpression has been detected in PDAC, breast cancer, and ovarian cancer by gene profiling.[13] Furthermore, the overexpression of claudin 4 in pancreatic intraepithelial neoplasia and its relationship with intraductal papillary mucinous neoplasms (IPMNs) have also been reported.[14–16] However, the expression of claudin 4 in PDAC has not yet been examined immunohistochemically using EUS-FNA.

Maspin is a member of the serpin superfamily of serine proteinase inhibitors. Its expression has been reported by using immunohistochemical studies of PDAC and high-grade intraductal dysplasia.[17] The diagnostic utility of surgical pathology specimens and tissue cell blocks obtained by EUS-FNA has been reported for maspin as well as Ki-67 and p53.[8,9,17]

The S100P is a member of the S100 family of calcium-binding proteins and promotes cancer progression via specific roles in survival, cell proliferation, invasion, and metastasis through its extracellular functions.[6] The expression of S100P in PDAC has been examined in detail in surgical specimens and EUS-FNA samples.[6,9] However, the accuracy of these antibodies alone is not 100%, and studies that have specifically focused on distinguishing PDAC from BE are very limited.

Therefore, the aim of the present study was to assess immunostaining patterns and results using the antibodies described previously and identify the optimal antibody panel as an adjunctive diagnostic tool, particularly for a differential diagnosis between PDAC and BE including a benign pancreatic ductal epithelium and contaminated gastric/duodenal epithelium in EUS-FNA material.

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MATERIALS AND METHODS

Cytological Smears and Tissue Cell Blocks

Seventy-five cases underwent EUS-FNA for pancreatic lesions at Kyoto University Hospital between August 2010 and June 2014. The PDAC cases were confirmed by a histological diagnosis from the resected pancreas, and benign cases were clinically confirmed by imaging studies and/or the clinical course with a follow-up duration of more than 9 months. We performed rapid onsite evaluation for all EUS-FNA cases and confirmed the puncture site. The EUS-FNA was performed only for solid mass. Intraductal papillary mucinous neoplasms and mucinous cystic tumors showing cystic lesions on image analysis are excluded from the subject for EUS-FNA. A 22-gauge needle was used for EUS-FNA. Samples were obtained and prepared for Diff-Quik stain for rapid on-site cytology to assure the quantity and quality of the material, whereas the remainder was wet-fixed for Papanicolaou staining. In addition, tissue fragments were fixed in 10% neutral-buffered formalin, embedded in paraffin, and then processed as a routine tissue block. Tissue sections from the cell block were stained with hematoxylin-eosin (HE) and used for immunohistochemistry. We prepared the cell blocks for all of the 75 EUS-FNA cases.

The study was approved by the Institutional Review Board of the Committee for Human Right in Research of Kyoto University Hospital.

Tissue Microarrays

Our tissue microarray (TMA) series consisted of PDAC cases (n = 53) and BE including benign pancreatic ducts (n = 10), benign gastric mucosa (n = 11), and benign duodenal mucosa (n = 12).

The TMA blocks were constructed from archival, formalin-fixed, and paraffin-embedded tissues. The diameter of all cores was 2 mm.

Immunohistochemical Analysis

Immunohistochemical staining was performed using paraffin-embedded cell blocks.

We selected claudin 4 (clone 3E2C1, 1:100; Invitrogen Corporation, Carlsbad, Calif), EZH2 (clone D2C9, 1:50; Cell Signaling Technology, Danvers, Mass), Ki-67 (clone MIB-1, 1:300; Dako North America, Inc, Carpinteria, Calif), maspin (clone G167–70,

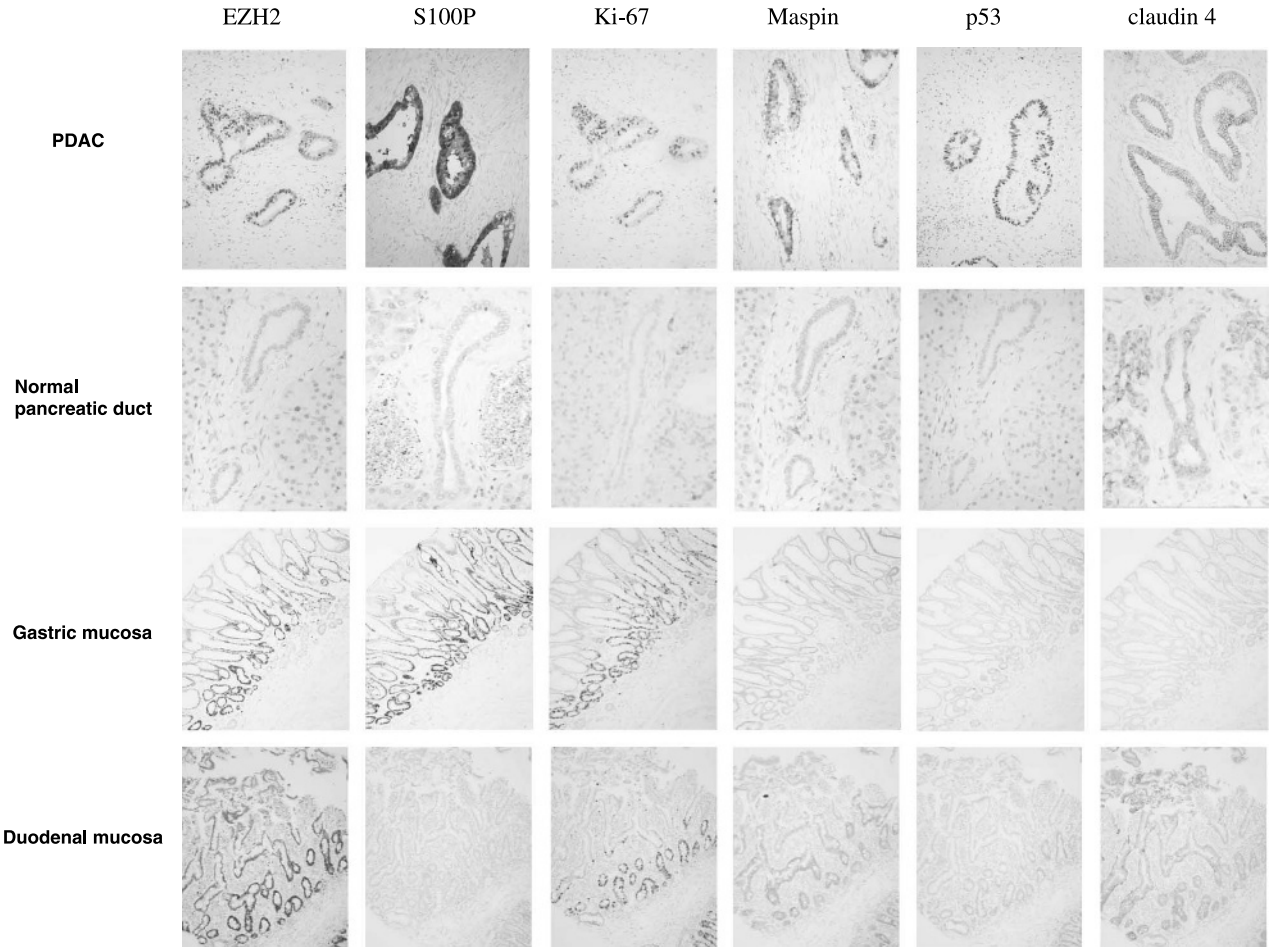


FIGURE 1. Immunostaining of PDAC, benign pancreatic duct, and gastric and duodenal mucosa with 6 markers (EZH2, S100P Ki-67, maspin, p53, and claudin 4). EZH2 and Ki-67 exhibit nuclear staining in PDAC and gastric/duodenal mucosa and are negative in the benign pancreatic duct. S100P showed nuclear and cytoplasmic staining in PDAC and the gastric mucosa and is negative in the benign pancreatic duct and duodenal mucosa. p53 showed nuclear staining in PDAC but is negative in the nonneoplastic epithelium of any type. Maspin showed nuclear and cytoplasmic staining in PDAC and a few cases of the duodenal mucosa and is negative in the benign pancreatic duct and gastric mucosa. Claudin 4 showed circumferential staining in PDAC, no staining in the gastric mucosa, and discontinuous staining in the benign pancreatic duct and duodenal mucosa.

1:1000; Becton Dickinson Immunocytometry Systems, San Jose, Calif), p53 (clone DO-7, 1:200; Dako North America, Inc), and S100P (clone 16/f5, 1:100; Ventana Medical Systems, Tucson, Ariz) as diagnostic markers. Immunostaining on cell blocks using the aforementioned antibodies was performed on the Ventana BenchMark URTLA instrument (Ventana Medical Systems). Nuclear staining for EZH2, Ki-67, and p53; membranous staining for claudin 4; membranous and cytoplasmic staining for maspin; and nuclear and cytoplasmic staining for S100P were regarded as positive. In the present study, immunostaining results for all but claudin 4 were scored as 0 (<5% of cells stained), 1+ (5%–25%), 2+ (26%–50%), 3+ (51%–75%), and 4+ (>75%). Regarding the membranous staining of claudin 4, scores were graded as 0 (no staining), 1+ (weak discontinuous staining), 2+ (weak circumferential staining), and 3+ (strong discontinuous or circumferential staining). Discordant cases were reviewed by 3 pathologists (S.M., T.S., and H.H.) using a multiheaded microscope, and consensus was reached.

P53 Large-Section Validation

To evaluate the p53 heterogeneity of BE, we performed p53 immunostaining on specimens of resected material, which was also used in TMA. Five blocks of different PDAC cases including benign pancreatic ducts and gastric and duodenal mucosa were selected. We selected 5 spots from each section and counted cells exhibiting positivity for p53.

Statistical Analysis

We used the receiver operating characteristic (ROC) curve to determine an appropriate cutoff point to discriminate between PDAC and BE. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 3.2.1, Vienna, Austria).[18]

Classification Tree

A classification tree was constructed using the Classification And Regression Trees (CART) method (Salford Predictive Modeler v7.0; Salford Systems, San Diego, Calif) based on the results of TMAs. We used GINI splitting criterion to measure quality of a split. Briefly, the distinction between PDAC and BE was set as the target, and the 6 antibodies were used as predictors. Parent node minimum cases were set as 10, and terminal node minimum cases as 1. The minimum-cost tree, that is, with the lowest level of classification errors, was the best tree. A 10-fold cross validation was used for testing.

RESULTS

Immunohistochemical Staining Patterns on TMAs

A photomicrograph of each marker in PDAC and BE on TMAs is shown in Figure 1. The ROC analysis revealed a cutoff point of 1+ for claudin 4, EZH2, Ki-67, p53, and S100P and 2+ for maspin (Fig. 2 and Table 1). The area under the curve (AUC) represents an optimal summary statistic for comparing sensitivities and specificities. Therefore, the cutoff values, sensitivities, and specificities for each marker were estimated at the highest AUC. Table 2 summarizes the staining results for 6 markers: claudin 4, EZH2, Ki-67, maspin, p53, and S100P.

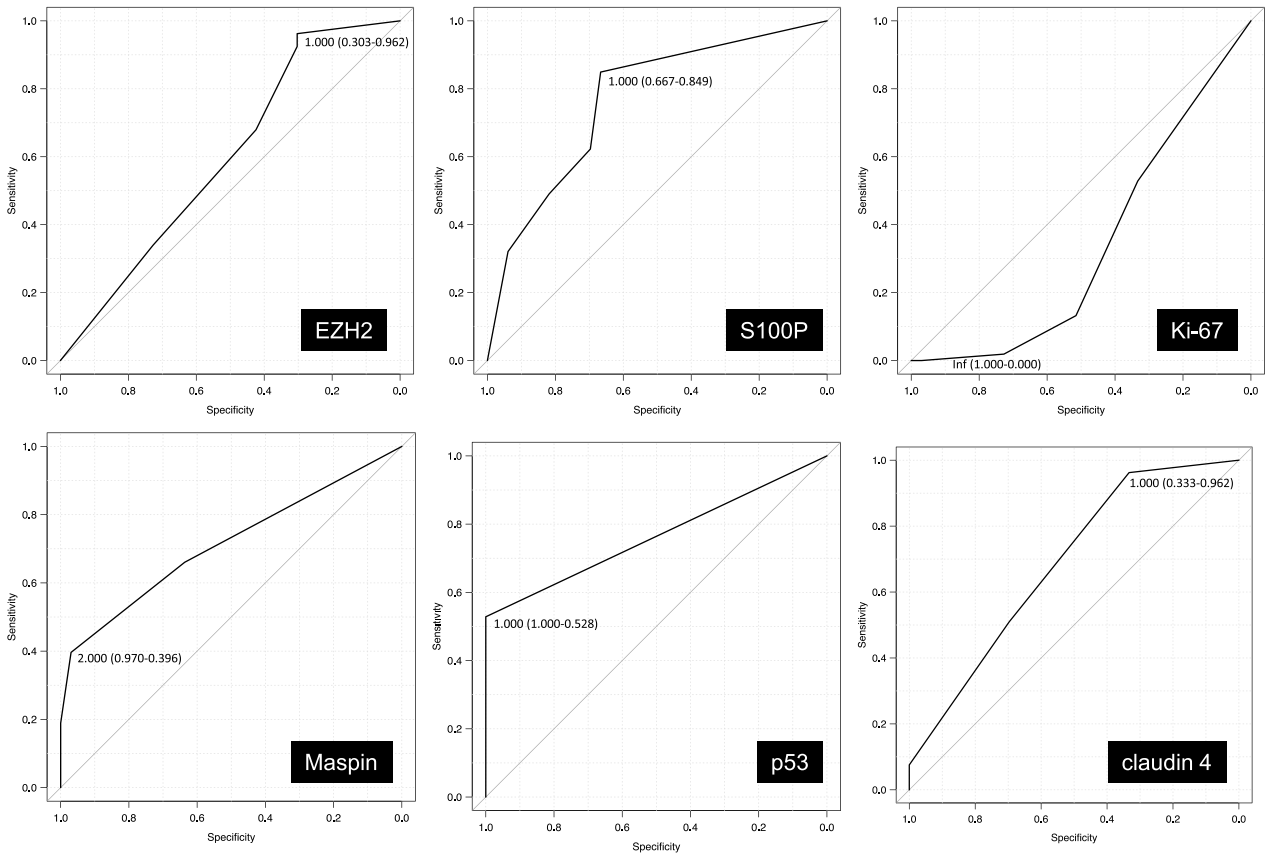


FIGURE 2. The ROC curve analysis for each of the 6 antibodies evaluated.

TABLE 1. Predictive Value of Each Marker

	Cutoff Value	Specificity	Sensitivity	AUC	95% CI
p53	1+	1	0.528	0.764	0.696–0.832
Maspin	2+	0.97	0.396	0.713	0.616–0.81
S100P	1+	0.667	0.849	0.765	0.662–0.868
claudin 4	1+	0.333	0.962	0.683	0.572–0.794
Ki-67	1+	0.333	0.528	0.333	0.211–0.455
EZH2	1+	0.303	0.962	0.595	0.469–0.721

CI indicates confidence interval.

We observed the following features in the BE: (1) A score of more than 0 for p53 was not observed in the BE of any type; (2) Scores of more than 0 for S100P were noted in some benign gastric epithelium; (3) Scores of more than 0 for claudin 4 or maspin were observed in some benign pancreatic ductal and duodenal epithelium; and (4) Scores of more than 0 for EZH2 and Ki-67 were observed in all types of epithelium.

Classification Tree

A CART analysis was performed to select the best subset among the 6 antibodies tested for discriminating PDAC from BE. The analysis showed 4- (using p53, S100P, and claudin 4) and 5-node (using p53, S100P, claudin 4, and maspin) classification trees (Fig. 3a, b upper). Prediction and misclassification for each node were also shown (Fig. 3a, b lower). In PDAC cases, the error rate of the 4- and 5-node classification trees was 3.8%. Two misclassified PDAC cases were apparently malignant both on cytology and cell block preparations.

In benign cases, the 4-node classification tree had a lower error rate than that of the 5-node tree (0% vs 3.8%).

Final Diagnosis and Cytological Diagnosis of EUS-FNA Materials

In histological diagnoses of the resected pancreas, all 53 cases were diagnosed as PDAC, and the 22 benign cases included 14 benign pancreatic ducts, 5 gastric mucosa, and 3 duodenal mucosa.

We used 4 categories, benign, atypical, suspicious, and malignant, as the reporting system of EUS-FNA. The original cytological diagnoses of 48 of 53 PDAC cases were malignant, with 1 and 4 cases being diagnosed as atypical and suspicious, respectively. All 22 benign cases were benign (Table 3).

Diagnostic Value Using the Classification Tree in TMA and EUS-FNA Specimens

The sensitivity and specificity of the classification tree for the diagnosis of PDAC were 96.2% and 100% in TMAs and 100% and 95.5% in EUS-FNA, respectively (Table 4). Although we originally achieved higher sensitivities and specificities in cytology and cell blocks in EUS-FNA, sensitivities and specificities of 100% were observed in combination with the classification tree (Table 5).

One case of PDAC with a cytological diagnosis of "atypical" included only a few clusters of 1 cell block preparation and was positive for p53 (Fig. 4).

p53 Large-Section Validation

The positivity of p53 in the resected specimen for the benign pancreatic ducts and gastric and duodenal mucosae was less than 5% in all spots, and these results were the same as those of TMAs (Table 6).

DISCUSSION

Although EUS-FNA is an excellent tool for diagnosing pancreatic cancer, false negatives and a cytological diagnosis of "atypical" remain issues because of the underestimation of well-differentiated adenocarcinoma and/or cases with little content. These cases are difficult to distinguish from contamination by benign gastric/duodenal mucosa or pancreatic cells by cytology only, and we need to evaluate cytological and cell block preparations together for EUS-FNA.[19]

Although many antibodies have been investigated for PDAC in EUS-FNA samples, previous studies that have focused on the diagnostic pitfalls of contamination of gastric/duodenal mucosa are very limited. Therefore, further studies are needed to identify useful markers for distinguishing PDAC from BE.

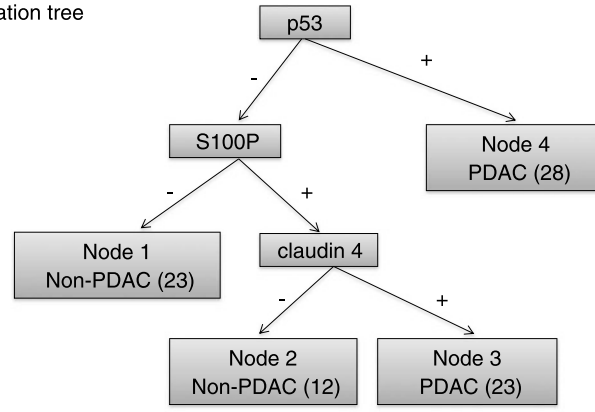
We herein analyzed 6 proteins, which have been reported to be overexpressed in PDAC. S100P, maspin, p53, and Ki-67 are recognized as good markers for diagnosing PDAC in cell block specimens obtained by EUS-FNA.[8,9] Liu et al[9] demonstrated strong or diffuse staining (>75% or 51%-75% of tumor cells stained) for S100P and maspin in all 44 EUS-FNA specimens from PDAC. However, they also revealed that the expression of S100P was frequently positive in the gastric mucosa, and maspin was expressed in the normal gastric antral and fundic mucosa as well as duodenal mucosa.

Jahng et al[8] reported that p53 and Ki-67 have the potential to improve the sensitivity of EUS-FNA in diagnosing PDAC. In their study, positive staining of more than 50% of cells was considered to be malignant, and 14/49 and 20/49 PDAC cases were positive for p53 and Ki-67, respectively.[8] However, we considered that this cutoff point (50%) was relatively high on the limited amount of tissue collected from EUS-FNA, and additional studies based on ROC analyses are needed to determine the cutoff levels for p53 in our study. Few studies have been conducted on EZH2 and claudin 4 in PDAC. Gao et al[11] reported the immunohistochemical positivity of EZH2 in EUS-FNA in 31/38 samples of PDAC. Our preliminary study revealed that EZH2 was expressed in the gastric mucosa. In previous studies, the cutoff points of the markers differed from each other, and immunostaining patterns in all contaminated epithelial samples (pancreatic ducts and gastric/duodenal mucosa) were not analyzed. Furthermore, the

TABLE 2. Immunohistochemical Staining of PDAC and Benign Epithelia in TMAs

	EZH2 (%)	S100P (%)	Ki-67 (%)	Maspin (%)	p53 (%)	Claudin 4 (%)
PDAC (n = 53)	51/53 (96)	45/53 (85)	28/53 (53)	21/53 (40)	28/53 (53)	51/53 (96)
BE (n = 33)	23/33 (70)	11/33 (33)	22/33 (67)	1/33 (3)	0/33 (0)	22/33 (67)
Pancreatic duct (n = 10)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	10/10 (100)
Gastric mucosa (n = 11)	11/11 (100)	11/11 (100)	10/11 (91)	0/11 (0)	0/11 (0)	0/11 (0)
Duodenal mucosa (n = 12)	12/12 (100)	0/12 (0)	12/12 (100)	1/12 (8)	0/12 (0)	12/12 (100)

4 Nodes classification tree

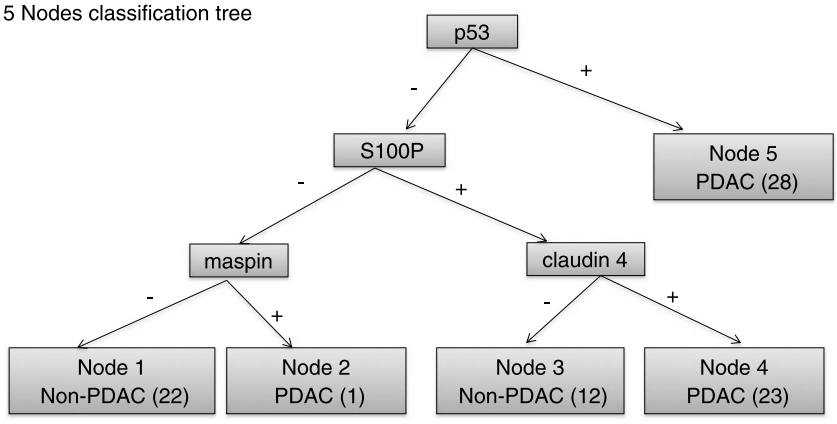


Prediction and misclassification for 4 nodes classification tree

		Proposed class			
Actual class	Total cases	PDAC N=51	Non-PDAC N=35	Misclassified	% error
PDAC	53	51	2	2	3.8
Benign	33	0	33	0	0

A

5 Nodes classification tree



Prediction and misclassification for 5 nodes classification tree

		Proposed class			
Actual class	Total cases	PDAC N=52	Non-PDAC N=34	Misclassified	% error
PDAC	53	51	2	2	3.8
Benign	33	1	32	1	3

B

FIGURE 3. The classification tree proposed by the CART method. Four- (using p53, S100P, and claudin 4) (A) and 5-node (using p53, S100P, claudin 4, and maspin) (B) classification trees were shown with prediction and misclassification for test data.

TABLE 3. Final Diagnosis and Cytological Diagnosis of EUS-FNA Materials

	Cytological Diagnosis			
Final Diagnosis	**Benign**	**Atypical**	**Suspicious**	**Malignant**
Pancreatic ductal adenocarcinoma (n = 53)	0	1	4	48
BE (n = 22)	22	0	0	0
Benign pancreatic duct (n = 14)	14	0	0	0
Gastric mucosa (n = 5)	5	0	0	0
Duodenal mucosa (n = 3)	3	0	0	0

use of only 1 marker may lead to false-positive results or a negative diagnosis. Therefore, to differentiate PDAC from benign pancreatic duct and contamination of the gastric/duodenal mucosa, we investigated several markers and proposed the combination

TABLE 4. Diagnostic Value of the 4-Node Classification Tree in TMAs and EUS-FNA Samples

	TMAs	**EUS-FNA**
Sensitivity (%)	96.2	100
Specificity (%)	100	95.5

TABLE 5. Diagnostic Accuracies of Cytology, Cell Blocks, and Immunocytochemistry, Alone or in Combination, in the Detection of Pancreatic Ductal Adenocarcinoma

	Sensitivity (%)	Specificity (%)
Cytology	90.6%	100.0%
Cell block	73.6%	100.0%
Cytology + cell block	96.2%	100.0%
Cytology + cell block + 4-node classification tree	100.0%	100.0%

of useful markers and a classification tree for diagnosing PDAC. Use of CART methods seems a reasonable way for constructing an optimized immunostaining panel. However, final diagnosis should be made by overall assessment because in this study, there were a couple of misclassified cases by CART methods alone.

Our immunostaining results from TMA sections indicated that p53 had the ability to distinguish PDAC from all samples contaminated by BE; however, its sensitivity and specificity were 53% and 100% by itself. The other markers did not differentiate PDAC from BE when used alone. Therefore, we derived a classification tree for diagnosing PDAC using CART software on the basis of immunohistochemical results from TMAs. As shown in

TABLE 6. p53 Heterogeneity on Large Sections (p53 Positivity, %)

Spot #	1	2	3	4	5
Pancreatic duct					
Case 1	2.99	3.47	0.00	3.13	0.32
Case 2	0.45	2.37	0.85	0.05	0.00
Case 3	2.56	1.22	0.55	0.03	0.20
Case 4	0.22	0.00	0.00	1.39	0.00
Case 5	0.00	0.00	0.00	0.00	0.87
Gastric mucosa					
Case 1	2.81	2.68	0.20	0.05	0.69
Case 2	0.00	0.00	0.00	0.00	0.00
Case 3	0.09	0.13	0.00	0.00	0.00
Case 4	0.18	0.02	0.06	0.16	0.08
Case 5	0.12	0.45	0.98	0.19	0.18
Duodenal mucosa					
Case 1	0	1.42	0.93	0.50	1.03
Case 2	0.27	0.00	0.00	0.25	0.09
Case 3	1.14	0.73	2.00	1.83	0.00
Case 4	0.21	0.96	1.32	1.43	0.92
Case 5	0.00	0.18	2.98	2.15	0.32

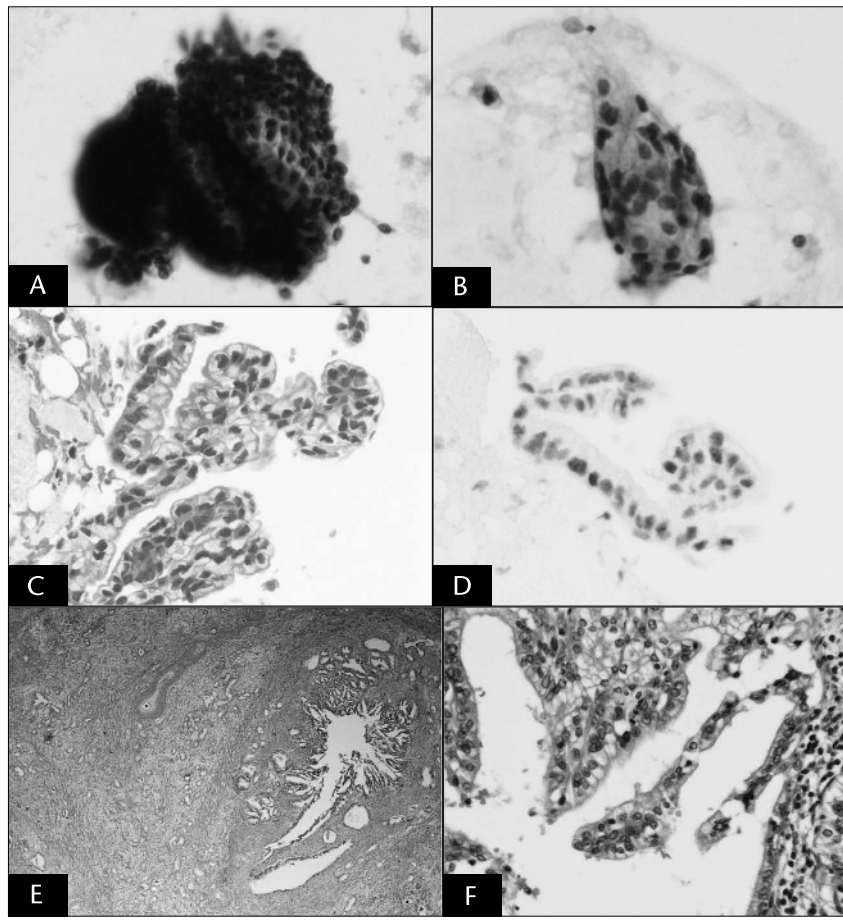


FIGURE 4. The PDAC diagnosed as atypical on cytology ([A] Papanicolaou 200×; [B] Papanicolaou 400×). The cluster of adenocarcinoma cells on a cell block ([C] HE 400×) was positive for p53 ([D] immunostain 400×). Resected pancreas tumor showing moderately to poorly differentiated PDAC ([E] HE 40×). High-power magnification of (E) ([F] HE 400×).

Figure 3, 4- and 5-node classification trees were provided by a CART analysis, and we also investigated the error rates of the 2 classification trees. Because the 5-node classification tree had 1 misclassification case in *benign*, we set the 4-node classification tree as the best minimal combination of antibodies for diagnosing PDAC. One interesting result of the present study was that the cutoff point for detecting p53 in PDAC was 5%. The cutoff point for p53 based on an ROC analysis was 5%, which was lower than that of a previous study on EUS-FNA that used p53 as a marker.[8] The lower positivity of p53 as evidence of PDAC may be related to premature senescence.

Premature senescence in response to abnormal mitogenic signaling is a mechanism underlying tumor suppression. Serrano et al[20] showed that the expression of oncogenic *ras* in primary human or rodent cells resulted in permanent G1 arrest, and this was accompanied by the accumulation of p53 and p16. They also demonstrated that senescence induced by oncogenic *ras* was indistinguishable from cellular senescence.[20] The *KRAS* is mutated in most cases of PDAC.[21] In the progression of oncogenic KRAS-induced carcinogenesis in the pancreas, the activity of the tumor suppressor p53 is promoted in oncogenic-induced senescence in which *p53* is genotypically the wild type.[22]

In the present study, the expression of p53 allowed for the detection of the mutant and wild-type forms of the p53 protein. However, the relationship between p53 immunohistochemistry and the *p53* mutation status has been controversial.[23] The immunohistochemical overexpression of p53 may closely correlate with the p53 mutation. The lower positivity of p53 may reflect the accumulation of wild-type p53 interpreted as premature senescence. In this case, the normally low level of p53 is elevated by cellular stress such as an oncogenic insult.[20] Therefore, the cutoff point of 5% for p53 in the present study may be valid for distinguishing PDAC from BE.

Furthermore, p53 heterogeneity is an important issue when adopting a lower cutoff point of p53 in the diagnosis of PDAC in EUS-FNA specimens because p53 may not be expressed in a fraction of the tissue. Because we only punched out 2 tissue cores from each block, TMAs contained only a limited amount of tissue. To assess the difference in p53 positivity in BE (benign pancreatic, gastric, and duodenal mucosae) between resected specimens and TMAs, we analyzed the p53 positivity of 5 spots from each resected specimen. Our results revealed that all cases of p53 positivity were less than 5%. This result was the same as that of TMAs and indicated that cases with more than 5% positivity for p53 are regarded as PDAC and also that a lower cutoff point may be used for EUS-FNA samples.

Among the 53 PDAC cases in the present study, there was only one atypical case on cytology (Fig. 4). This case was diagnosed as moderately to poorly differentiated PDAC by a histological diagnosis of the resected pancreas. In cytological smears, this case was confused with the gastrointestinal epithelium because clusters of neoplastic cells showed flat sheets, and there was no remarkable cellular atypia. The cell block preparation contained only 1 cluster of atypical cells, that is, positive staining for p53, and may be diagnosed as PDAC using an HE-stained cell block preparation and immunocytochemistry.

Although we investigated useful markers for diagnosing PDAC, IPMNs are also difficult to distinguish from a nonneoplastic gastric mucinous epithelium based on cytological features alone.[24]

In Western countries, EUS-FNA is preferable for histological and/or cytological diagnoses of IPMNs. Data to support the utility of EUS-FNA in pancreatic cystic neoplasms are limited, and the positive predictive value of an FNA diagnosis of IPMN and the sensitivity of FNA cytology for IPMNs is not yet established. Sedlack et al[25] investigated 18 cases and revealed that whereas specificity was high (100%), the sensitivity of cytology was only 27% for diagnosing IPMNs. In a recent single-center study of 141 cysts, cytology findings were highly specific (51/53, 96%), whereas sensitivity was moderate (61/141, 43%).[26] In Japan, endoscopic retrograde cholangiopancreatography is recommended for a cytological diagnosis of IPMNs instead of EUS-FNA because of the risk of the dissemination of tumor cells or pseudomyxoma peritonei after EUS-FNA. Therefore, we did not investigate IPMN cases in this study. However, if an EUS-FNA diagnosis of IPMNs is required to improve the poor sensitivity associated with diagnosing IPMN by EUS-FNA, further studies on immunohistochemical methods to distinguish PDAC from benign gastric mucosa are needed.

In summary, to the best of our knowledge, we are the first to report a useful immunostaining panel for distinguishing PDAC from all contaminated BE in the English-language literature.

An accurate diagnosis of PDAC on EUS-FNA cell blocks is sometimes difficult because of the limited amount of tissue, particularly in cases of a small amount of tissue and well-differentiated PDAC. Contamination of gastric/duodenal epithelium is a diagnostic pitfall in the diagnosis of PDAC. In this case, the classification tree using 3 antibodies (p53, S100P, and claudin 4) proposed herein seems to successfully distinguish PDAC from the accompanying contaminated BE in EUS-FNA samples.

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