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Original Article

Diagnostic and therapeutic implications of a novel immunohistochemical panel detecting duodenal mucosal invasion by pancreatic ductal adenocarcinoma

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Abstract: Background: We investigated a series of pancreaticoduodenectomy and duodenal biopsies with a panel of immunohistochemical markers to identify duodenal mucosal invasion by pancreatic ductal adenocarcinoma (PDAC), including markers of poor prognosis and targets of promising novel immunotherapies. Materials and Methods: Eighteen consecutive pancreaticoduodenectomy specimens with duodenal mucosal invasion by PDAC were examined for expression of MUC1, MUC4, MUC5AC, MUC6, mesothelin, MUC2, CDX2, and DPC4 on formalin-fixed, paraffin-embedded sections of duodenal-ampullary-pancreatic junctions. Expression of all but MUC6 was also assessed in duodenal biopsies from 12 patients with duodenal mucosal invasion by PDAC. Results: The duodenal mucosa expressed MUC1 (crypts), MUC2 (goblet cells), MUC6 (Brunner glands), CDX2, and DPC4. PDACs in the duodenal mucosa from the resection (n=16-18) and biopsy (n=12) specimens were marked as follows: MUC1 100% (30/30), MUC4 83% (24/29), MUC5AC 83% (25/30), mesothelin 82% (23/28), MUC2 7% (2/30), and CDX2 36% (10/28). Loss of DPC4 expression was seen in 16 of 29 (55%) cases. Reactive mucosa adjacent to PDAC expressed MUC4, MUC5AC and mesothelin in 65% (17/26), 19% (5/27), and 19% (5/26) of cases, respectively. While MUC5AC and mesothelin had high diagnostic accuracy for detection of PDAC, MUC2, CDX2 and DPC4 expression demonstrated negative correlation with PDAC, with absent expression being highly specific for PDAC. Conclusion: Immunohistochemical labeling for PDAC biomarkers may aid the diagnosis of PDAC in duodenal biopsy, especially in situations where diagnosis of a pancreatic mass is challenging.

Keywords: Pancreatic ductal adenocarcinoma, duodenal mucosal invasion, immunohistochemistry

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer deaths in the United States with a 5-year overall survival rate less than 6% [1, 2]. The best chance for cure is surgical resection; however, less than 20% of patients are eligible for this procedure due to advanced disease at diagnosis [2]. Even in this select surgical population, the 5-year overall survival is only 18-24% [1, 3, 4]. These facts underscore the significant medical problem presented by this disease.

The initial workup for obstructive jaundice and/or a pancreatic mass may include endoscopic duodenal biopsy. The pathologic diagnosis of PDAC on such endoscopic biopsies is challenging at best. Common pitfalls include overdiagnoses of reactive epithelial changes, or underdiagnoses of histologically bland but biologically aggressive PDAC colonizing duodenal mucosa. In cases of apparently benign duodenal biopsies on hematoxylin and eosin stain, an immunohistochemical panel identifying duodenal mucosal involvement by PDAC may increase the sensitivity of detection with accurate diagnosis, thus accelerating a potential curative resection.

Many secreted and plasma membrane-bound molecules are over-expressed in PDAC. These include mesothelin, growth factor receptors, and a number of mucins (MUC1, MUC4, and

Table 1. Primary antibodies comprising the immunopanel

Antibody (clone)	Dilution	Species	Commercial source	Catalog#
MUC1 (VU4H5)	1:100	mouse	Cell Signaling	4538
MUC2 (Ccp58)	1:300	mouse	Abcam	ab49460
MUC4 (8G7)	10 μg/mL	mouse	Santa Cruz Biotech	sc-53945
MUC5AC (Ab-1)	1:300	mouse	Thermo Scientific	MS-145
MUC6 (N.A.)	1:200	mouse	Novacastra	NCL-MUC6
CDX2	1:400	rabbit	Cell Signaling	3977
DPC4 (SMAD4)	1:50	mouse	Santa Cruz Biotech	sc-7966
Mesothelin	1:200	rabbit	Abcam	ab93620

MUC5AC) [5-18]. Mucins are highly complex molecules with extensive O-linked glycosylation of their extracellular domain, and may display specific epitope structures during the development and progression of PDAC [19]. Several monoclonal antibodies (MAbs) have been developed to label specific mucin epitopes that display altered distribution as a consequence of neoplasia [20, 21]. Loss of expression of tumor suppressor genes is also common in PDAC. For example, loss of the tumor suppressor gene *DPC4/SMAD4* occurs in approximately half of all cases of PDAC, and is associated with aggressive behavior and widespread tumor metastases [22-24]. Additionally, the majority of PDAC specimens do not have an intestinal phenotype, and markers of intestinal differentiation such as MUC2 and CDX2 may help differentiate PDACs from adjacent non-neoplastic duodenal epithelium or primary duodenal adenocarcinoma [25-27].

In this study, we examined 18 consecutive pancreaticoduodenectomy specimens and 12 duodenal biopsies showing duodenal mucosal invasion by PDAC by immunohistochemical labeling to develop a strategic panel of biomarkers for improved detection of PDAC in the duodenal mucosa.

Materials and methods

Tissue specimens

This HIPAA-compliant study was approved by the Institutional Review Board at Vanderbilt University. One hundred fifty patients with PDAC who underwent pancreaticoduodenectomy at Vanderbilt University Medical Center from 01/2005 to 12/2009 were identified, among which 18 (12%) demonstrated duodenal mucosal invasion by PDAC. Formalin-fixed, paraffin-embedded sections containing the duodenal-ampullary-pancreatic junctions from these 18 cases were used to perform immunohistochemical studies. Twelve duodenal biopsies from patients with duodenal mucosal invasion by PDAC were also identified from 01/2009 to 12/2011 and subjected to immunohistochemical studies.

Immunohistochemistry

Immunohistochemical labeling for MUC1, MUC2, MUC4, MUC5AC, MUC6, mesothelin, CDX2, and DPC4 was performed on 5 μm-thick formalin-fixed, paraffin-embedded sections of duodenal-ampullary-pancreatic junctions and duodenal biopsies as described below (MAb clones, their commercial source, and dilution employed for these studies are provided in **Table 1**). Biomarker expression patterns were independently recorded by three pathologists (SCW, PG, CS) and were compared with tumor grade. All stains were recorded as follows: diffuse, ≥25%; focal, 1%-25%; negative, <1%. The intensity of the stain was graded as strong, moderate or weak.

Unstained slides were deparaffinized by routine methods with antigen retrieval performed by heating the slides in a pH 6.0 citrate buffer at 100°C for 20 minutes followed by a 10-minute cooling to room temperature. All slides were then quenched with 0.03% H_2O_2 with sodium azide for 5 minutes. Slides were blocked for 20 minutes with serum-free protein block (Dako, Carpentaria, CA), followed by application of primary antibody (**Table 1**) for 60 min (except for anti-MUC1 MAb which was left on the tissue sections overnight). MAbs were detected by incubation with Envision+HRP-labeled polymer (Dako) for 30 minutes, followed by 5 minute incubation with DAB (Dako).

Statistics

A statistical comparison of biomarker expression was performed. For each biomarker, the labeling results of individual specimens were converted to either negative (0) or positive (1) values for benign duodenal tissue and duodenal mucosa involved by PDAC, with the data

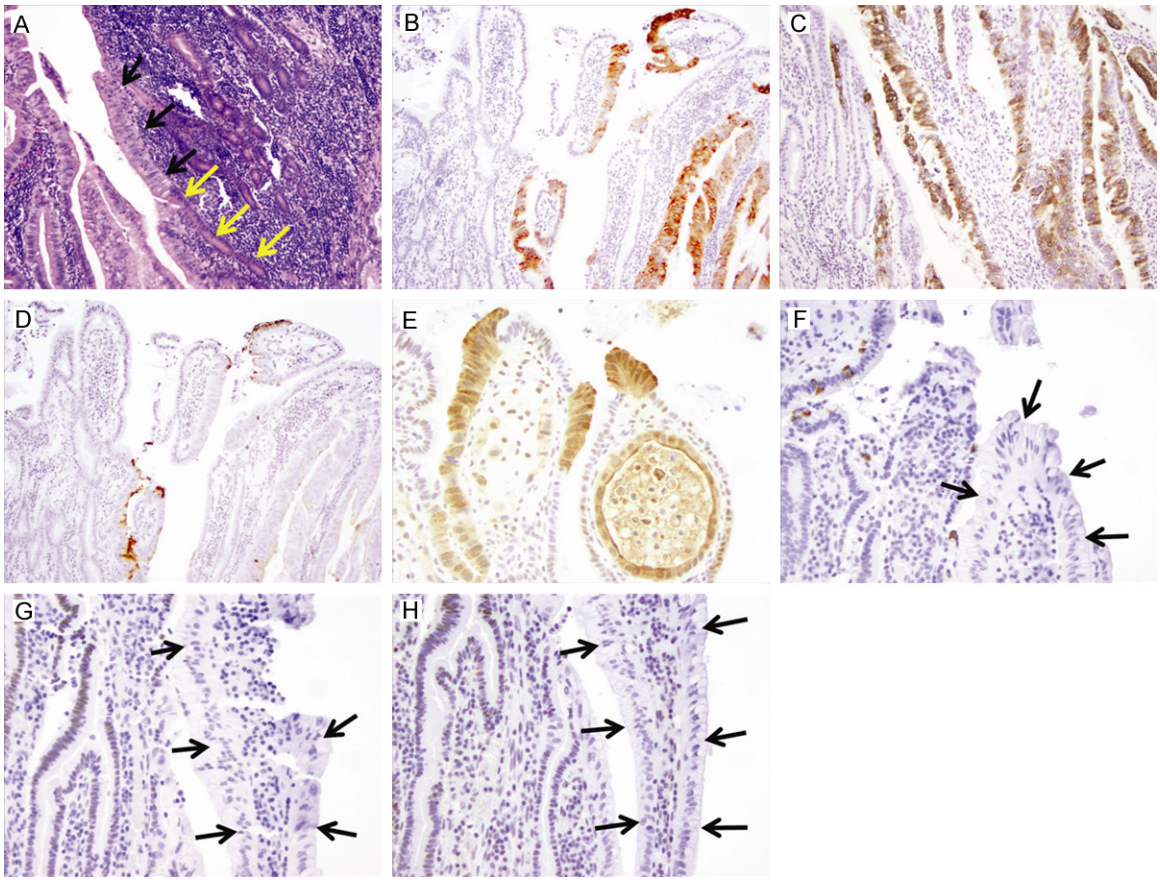

Figure 1. Duodenal mucosal involvement by pancreatic ductal adenocarcinoma (PDAC) in an epithelial colonization fashion (original magnification 200X). A. Hematoxylin and eosin stain showing duodenal epithelial cells replaced by malignant cells (Black arrows: malignant epithelium; Yellow arrows: normal epithelium); B. Diffuse strong MUC1 expression in PDAC; C. Diffuse MUC4 expression in PDAC; D. MUC5AC expression in PDAC; E. Diffuse mesothelin expression in PDAC; F. Lack of MUC2 expression in PDAC (Black arrows: malignant epithelium); G. No CDX2 expression in PDAC (Black arrows: malignant epithelium); H. Lack of DPC4 expression in PDAC (Black arrows: malignant epithelium).

analyzed by receiver-operating characteristic curves.

Results

Eighteen of 150 resected PDACs demonstrated duodenal mucosal involvement. These cases were graded as follows: well-differentiated (2/18, 11%), moderately differentiated (10/18, 56%), and poorly differentiated (6/18, 33%). Mucosal involvement manifested as malignant cells colonizing the duodenal epithelium (**Figure 1A**) and/or malignant glands infiltrating the mucosa (**Figure 2A**). The biomarker profiles were similar for both types of mucosal involvement (**Figures 1B-H** and **2B-H**), and are summarized in **Table 2** for benign duodenal mucosa and duodenal mucosal invasion by PDAC.

We first compared MUC1 expression in PDACs in the duodenal mucosa to that in benign duodenal mucosa using an antibody reactive with the VNTR (VU4H5). This MAb labeled background duodenal crypts and Brunner's glands, but rarely duodenal villi. Labeling for MUC1 in the duodenal mucosa progressed from positive in the deep crypts to negative in the villi. Eighteen of 18 (100%) PDACs in the duodenal mucosa labeled for MUC1, with 15 of them (83%) showing strong, diffuse MUC1 expression. MUC1 labeling abruptly transitioned from positive in PDACs to negative in benign duodenal villi, which made it recognizable from background duodenal epithelium (**Figure 1B**). In addition, compared to background duodenal crypts, PDAC was frequently more intensely labeled.

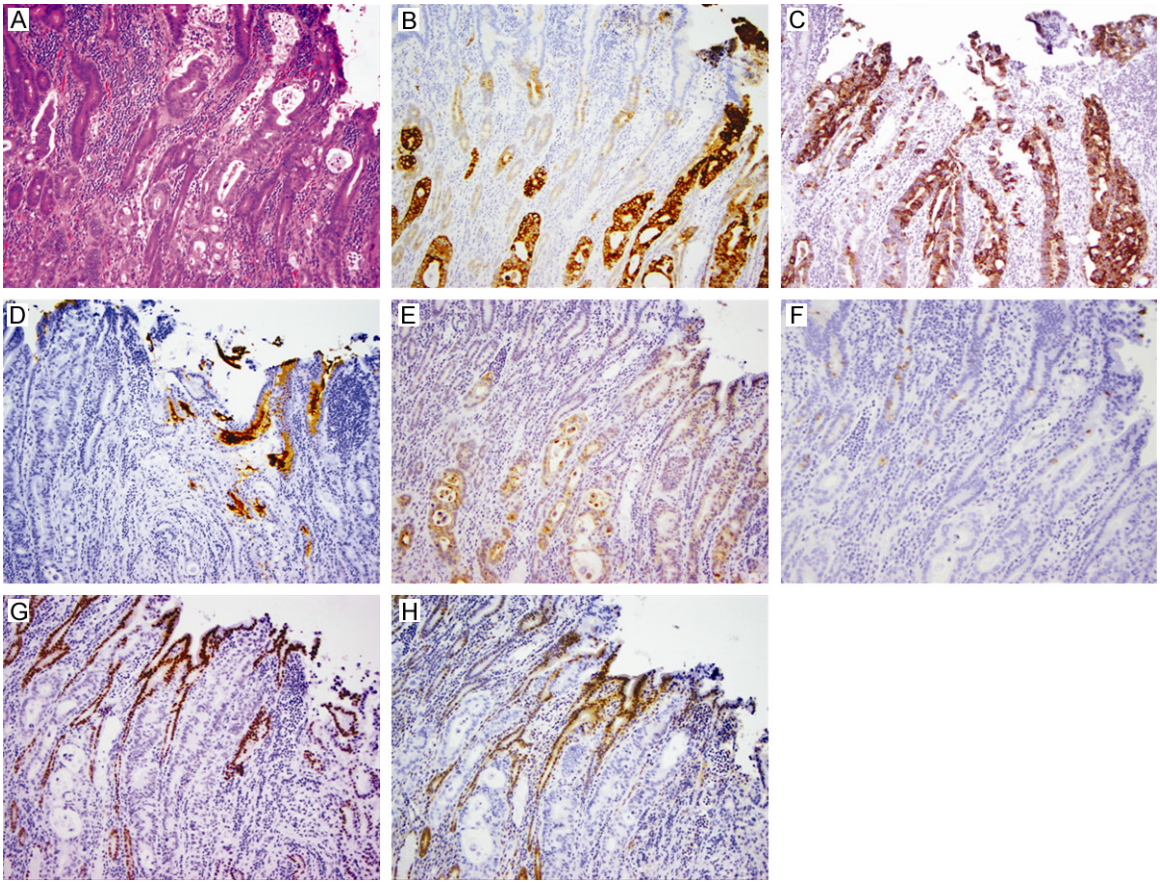

Figure 2. Duodenal mucosal involvement by pancreatic ductal adenocarcinoma in a glandular infiltrating fashion (original magnification 100X). A. Hematoxylin and eosin stain showing malignant glands in the duodenal mucosa; B. Diffuse strong MUC1 expression in malignant glands; C. Diffuse strong expression of MUC4 in malignant glands; D. Focal strong MUC5AC expression in malignant glands (note: MUC5AC-positive mucin in the glands); E. Diffuse mesothelin expression in malignant glands; F. Lack of MUC2 expression in malignant glands; G. No CDX2 expression in malignant glands; H. Loss of DPC4 in malignant glands.

Immunohistochemical labeling for MUC4 demonstrated that 82% (14/17) of PDACs expressed at least focal MUC4 (**Table 2**), 4 with focal expression and 10 with moderate to strong, diffuse expression (**Figures 1C** and **2C**). However, scattered MUC4 positive cells were also present in the reactive mucosa adjacent to PDAC in 8 of 17 (47%) cases studied.

The expression of MUC5AC in PDAC was also examined. All but one PDACs (17/18, 94%) were labeled with anti-MUC5AC antibody (**Table 2**, **Figures 1D** and **2D**). MUC5AC was not expressed by normal duodenal mucosa, but was expressed by gastric metaplasia. Duodenal gastric metaplasia is not uncommon in settings of chronic injury/inflammation. MUC5AC expression is, therefore, not entirely specific for PDAC. However, compared to its weak expression in the metaplastic mucosa, MUC5AC expression in PDAC was very strong in most positive cases, and thick MUC5AC positive mucin was frequently observed within cancerous glands.

MUC6 was frequently expressed by duodenal mucosa (94%), especially Brunner's glands, whereas only 39% (7/18) of PDACs expressed MUC6. Therefore, MUC6 appeared to be a poor marker for differentiation of PDACs from the duodenal mucosa.

Mesothelin is a biomarker that is highly expressed in PDAC [9, 10, 13]. Nearly all PDACs (94%) expressed this marker (**Figures 1E** and **2E**). While the labeling was mostly strong, and especially prominent in the luminal surface membranes of the tumor cells, four of them only had focal expression of mesothelin. In

Table 2. Expression of biomarkers in the duodenal mucosa and DMI-PDAC

	Resection		Biopsy		Overall	
	Duodenum	PDAC	Duodenum	PDAC	Duodenum	PDAC
MUC1	17/18 (94%)	18/18 (100%)	9/9 (100%)	12/12 (100%)	26/27 (96%)	30/30 (100%)
MUC4	8/17 (47%)	14/17 (82%)	9/9 (100%)	10/12 (83%)	17/26 (65%)	24/29 (83%)
MUC5AC	3/18 (17%)	17/18 (94%)	2/9 (22%)	8/12 (67%)	5/27 (19%)	25/30 (83%)
MUC6	17/18 (94%)	7/18 (39%)	-	-	17/18 (94%)	7/18 (39%)
Mesothelin	5/17 (29%)	15/16 (94%)	0/9 (0%)	8/12 (67%)	5/26 (19%)	23/28 (82%)
MUC2[a]	0/18 (0%)	17/18 (94%)	0/9 (0%)	11/12 (92%)	0/27 (0%)	28/30 (93%)
CDX2[a]	0/16 (0%)	11/16 (69%)	0/9 (0%)	7/12 (58%)	0/25 (0%)	18/28 (64%)
DPC4[a]	0/17 (0%)	8/17 (47%)	0/12 (0%)	8/12 (67%)	0/29 (0%)	16/29 (55%)

addition, the expression of mesothelin was also observed in reactive duodenal mucosa in some (29%) cases.

The expressions of two intestinal biomarkers, MUC2 and CDX2, in PDAC were also explored and compared to the benign mucosa. Background duodenal villi and crypts showed MUC2 labeling in goblet cells (100%), while 17/18 (94%) PDACs lacked the expression of this mucin (**Figures 1F** and **2F**). All duodenal epithelial cells expressed nuclear CDX2. In PDACs, 69% of cases did not express CDX2 (**Figures 1G** and **2G**). Therefore, lack of CDX2 and MUC2 expression may be useful as confirming evidence for detection/diagnosis of PDAC.

Loss of DPC expression is frequently seen in PDAC. Nuclear expression of DPC4 was present in normal duodenal mucosa, stromal and inflammatory cells. However, 47% PDACs did not express DPC4 (**Table 2**, **Figures 1H** and **2H**). For that reason, complete loss of DPC4 expression is highly specific for PDAC when compared to background benign mucosa; however, the sensitivity is low.

Since some of the biomarkers studied were only focally expressed in the resection specimens, their expression was also examined in duodenal biopsies from patients with known PDAC to determine the feasibility of using these biomarkers in biopsy specimens. Twelve patients were identified, including 1 with well differentiated, 6 moderately differentiated, 2 poorly differentiated, and 3 undifferentiated carcinomas. MUC1 was diffusely and focally expressed in 8 (66%) and 4 (34%) of the 12 cases, respectively. Similar to the findings from the resection specimens, the well to moderately differentiated carcinomas (**Figure 3A**) had diffuse, much stronger MUC1 labeling than background duodenal mucosa (**Figure 3B**). Therefore, although MUC1 expression is not specific, the intensity of MUC1 labeling might be helpful in distinguishing PDAC from benign duodenal mucosa.

Ten of the 12 (83%) cases expressed at least focal MUC4 in the biopsies, including 5 (42%) with diffuse, strong expression (**Figure 3C**). However, the adjacent duodenal mucosa also showed scattered positive cells. Thus MUC4 may not be suitable for differentiating PDAC from duodenal mucosa. MUC5AC was expressed in 67% (8/12) of the cases, and frequently was present within the malignant glands (**Figure 3D**). Due to the fact that MUC5AC is expressed by the mucosa with gastric metaplasia, the expression of MUC5AC was also not specific for PDAC. However, the intensity and the pattern (thick mucin gel within the glandular structures) of the stain may aid in distinguishing between PDAC and the adjacent mucosa.

MUC1, MUC4, and MUC5AC were expressed by all well to moderately differentiated PDACs in the duodenal biopsies. The expression profiles of each mucin appeared to correlate with grade of tumor differentiation, although expression of MUC4 has been reported to correlate with poor differentiation in PDAC [5, 28]. Mesothelin expression was seen in 67% of cases, but unlike the resection specimens, the biopsies did not show mesothelin expression in background duodenal mucosa (**Figure 3E**). Similar to the mucin markers, mesothelin expression was not detected in the 3 undifferentiated carcinomas.

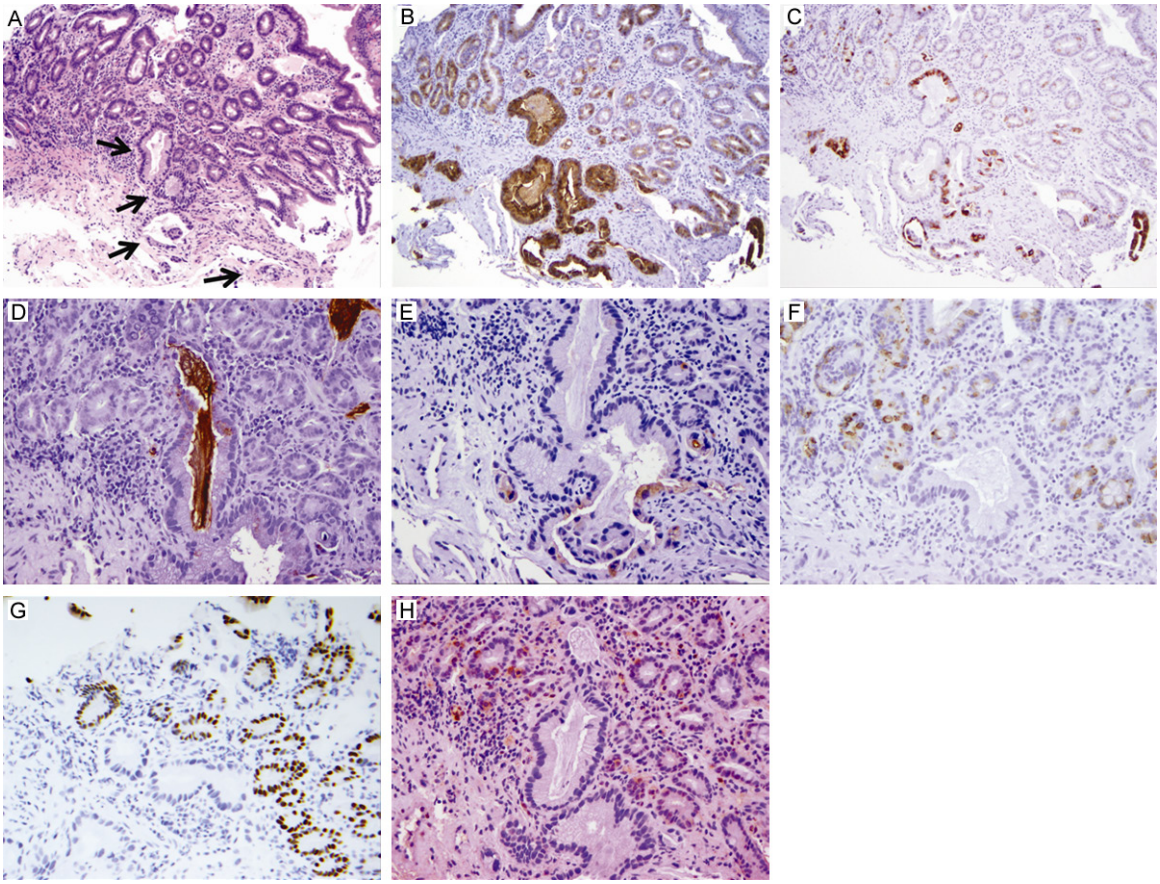

Figure 3. Duodenal mucosal involvement by pancreatic ductal adenocarcinoma (PDAC) in one duodenal biopsy. A. Hematoxylin and eosin stain showing malignant glands in the duodenal biopsy (original magnification 100X; black arrows: malignant glands); B. Diffuse strong MUC1 expression in malignant glands (100X); C. MUC4 expression in malignant glands (100X; note: scattered MUC4 expression in background duodenal mucosa); D. MUC5AC expression in malignant glands (200X; note: MUC5AC positive mucin in the glands); E. Focal mesothelin expression by malignant glands (200X); F. Lack of MUC2 expression in one large malignant glands (200X); G. No CDX2 expression in malignant glands (200X); H. Loss of DPC4 expression in malignant glands (200X).

MUC2 expression was seen in only one biopsy with features of mucinous (colloid) adenocarcinoma. Background duodenal mucosa demonstrated scattered MUC2 expressing goblet cells, but some cross sections of small duodenal crypts could be lack of goblet cells. Therefore, absent MUC2 expression in small glands may not correlate with invasive tumor glands. However, lack of MUC2 expression in large glandular structures may be suggestive of malignancy (**Figure 3F**). CDX2 (**Figure 3G**) and DPC4 (**Figure 3H**) were not expressed in 58% and 67% of the cases, respectively.

A statistical comparison of biomarker expression is presented in **Table 3**, sorted by diagnostic accuracy (highest to lowest). MUC2 as a single biomarker provided the highest diagnostic accuracy, but has a negative correlation with PDAC. In addition, as mentioned above, small duodenal crypts might be lack of goblet cells. Therefore, it is unlikely that this biomarker would prove useful as a stand-alone agent, but rather as a means to provide confirmation while another biomarker provides positive reaction with PDAC. Of those biomarkers with positive correlation to PDAC, MUC5AC and mesothelin were not statistically different ($P>0.05$).

Discussion

A biomarker panel comprised of well-known, reliable immunohistochemical markers (MUC1, MUC5AC, MUC2, mesothelin, and CDX2), and those with potential prognostic and/or theranostic significance (MUC1, MUC4, MUC5AC,

Table 3. Diagnostic performance of individual biomarkers sorted by diagnostic accuracy

	Sensitivity (%)	Specificity (%)	Diagnostic Accuracy (%)
MUC2[a]	93	100	97
MUC5AC	83	81	82
CDX2[a]	64	100	81
Mesothelin	82	81	81
DPC4[a]	55	100	78
MUC4	83	35	60
MUC1	100	4	54
MUC6	39	6	44

[a]These biomarkers have a negative correlation with PDAC.

and DPC4) was chosen for evaluation of PDAC [5, 14, 17, 28, 29]. Some of these biomarkers (MUC1, mesothelin) represent promising targets of immunotherapy through the use of radiolabeled antibodies or vaccines [30-39], while modulation of MUC4 expression lends increased sensitivity to front-line chemotherapy [40], and the expression of MUC1, MUC5AC and mesothelin provide insight into patterns of tumor development, spread, and aggression [7, 14, 41, 42]. Therefore, this immunohistochemical panel may offer a sensitive method of detection of PDAC with an immediate, multifaceted assessment of tumor behavior and susceptibilities. In addition, this panel may have potential to maximize the information derived from biopsies and better direct therapy in the initial workup of pancreatic cancer patients.

Five of the 8 biomarkers analyzed in this study are mucins. Two subfamilies of mucin species have been described: secreted and plasma membrane-bound. Membrane-bound mucins have a structure composed of an integral transmembrane domain, a short cytoplasmic tail, and an extracellular, juxtamembrane domain with homology to the epidermal-growth-factor (EGF) family [43]. A paracrine signaling pathway is implicated, with binding of EGF-like domains to EGF receptors, to regulate growth, motility, differentiation, inflammation, or other functions. One of the membrane-bound mucins, MUC1, is expressed early in the pancreatic ductal dysplasia-carcinoma sequence and is found in approximately 90% of PDAC cases [12, 15]. The expression of specific MUC1 structures/epitopes is associated with tumor progression and decreased cellular adhesion through cytoplasmic tail interaction with the β-catenin and MAPK pathways [35, 43]. MUC1 is also a receptor for myelin-associated glycoprotein on Schwann cells and oligodendroglia, which contributes to the extensive perineural invasion seen in the majority of PDAC specimens [44]. Additionally, the extracellular juxtamembrane domain interacts with the ErbB family [45], and activates ERK and Akt, promoting cellular proliferation [35]. *In vitro* studies have shown decreased proliferation and migration through decreased ERK phosphorylation with anti-MUC1 antibody treatment [35], which may represent a novel future immunotherapy.

Another member of the membrane-bound mucin subfamily, MUC4, is aberrantly expressed in precancerous pancreatic intraepithelial neoplasias (PanINs) and PDACs, and is not expressed in normal pancreas. Expression correlates with PanIN dysplasia [17], and with tumor grade [8, 28]. MUC4 contains an extracellular juxtamembrane domain EGF-like motif which interacts with HER2/neu (ErbB2) and shows sequence homology to heregulin [43]. Signaling is postulated to occur through p27 and MAPK pathways, and is associated with tumor growth, proliferation, resistance to apoptosis, survival, and tumor invasion/metastasis [8]. In a recent multivariate analysis, MUC4 was the only significant factor associated with a poor prognosis [8]. *In vitro* studies have shown that MUC4 downregulation improves gemcitabine response [40]. Our study showed no association of immunohistochemical expression with poor tumor grade, which may be reflective of a small sample size. Scattered MUC4 positive duodenal epithelial cells were seen and especially prominent in the area closely adjacent to PDAC. Although MUC4 is not specific for PDAC, identification of its expression may help choose a better treatment strategy for patients with PDAC in the future.

MUC5AC, a member of the secreted subfamily, is a gastric-type mucin expressed by normal gastric epithelium but not normal pancreas. Like MUC1, MUC5AC is expressed early in the pancreatic-ductal dysplasia sequence [7, 20]; however, unlike MUC1, it is expressed less frequently and shows loss of expression in high grade tumors [7, 14]. Some studies have demonstrated that expression is associated with a more favorable prognosis [7, 14], while others have shown an association with a poor prognosis [18, 41]. Loss of MUC5AC expression is

associated with lymphovascular invasion and lymph node metastasis [14], while xenograft studies have shown that MUC5AC expression may allow pancreatic cancer cells to evade immunosurveillance [19]. Recent studies show that MUC5AC expression is associated with decreased E-cadherin-dependent cell-cell adhesion and the β-catenin signaling pathway [41]. The association of MUC5AC staining intensity with tumor grade was observed in the present study, with undifferentiated carcinoma showing little to no expression.

Expression of MUC5AC was observed in the majority of PDAC. However, this biomarker was also expressed in gastric mucin cell metaplasia of the duodenal mucosa, a phenomenon following chronic stimulation due to inflammatory processes or malignant involvement. We observed that expression of the biomarker was weak in gastric mucin cell metaplasia, whereas the expression was often strong in PDAC. In addition, gastric mucin cell metaplasia is easily recognized on histologic ground and usually located at the surface of the mucosa. Therefore, immunohistochemical labeling for MUC5AC can still be a powerful tool to diagnose PDAC.

Mesothelin, a GPI-anchored protein whose function is unknown, but may mediate cell-cell adhesion, is a recently identified biomarker for PDAC and is not expressed in normal pancreas, although its expression in duodenal mucosa has been observed in some cases. Its expression is minimal in PanINs but is found in nearly all cases of PDAC [13, 46]. Studies of pancreatic fine needle aspirate biopsies demonstrate a high specificity for pancreatic cancer as compared with normal pancreas or reactive atypia [10, 16]. Due to its relative specificity for a small number of tumors (PDAC, ovarian carcinoma, and mesothelioma), it is a new target for immunotherapies, with some success [30-32, 34, 36]. Patients with PDAC may be chosen for mesothelin-targeting therapy based on IHC detection of its expression using duodenal biopsy, should PDAC be present.

Well to moderately differentiated PDAC may invade the duodenum, where tumor glands/cells can closely mimic reactive duodenal epithelium. Here we demonstrated that MUC5AC and mesothelin, having a positive correlation with the presence of PDAC, may be used to discriminate PDAC from duodenal epithelium. Confirmation may be demonstrated by use of a biomarker with negative correlation, that is, high expression within the background, histologically normal duodenal tissue, such as MUC2. In addition, the intensity and pattern of MUC1 may be useful to distinguish PDAC from background duodenal mucosa. However, morphology remains the most important part of diagnosis, and immunohistochemical results should be evaluated in context of morphology and the patient's clinical history.

In summary, detection of PDAC in biopsy specimens is a diagnostic challenge. When hematoxylin and eosin observations show morphologically suspicious glands/cells, immunohistochemical stains for these biomarkers, may be helpful in the diagnostic workup and provide timely information that can provide evidence of the tumor's biologic behavior for efficient therapeutic decision-making.

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Disclosure of conflict of interest

None.

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References

[1] Surveillance Epidemiology and End Results. SEER Fact Sheets 2012.

[2] Vincent A, Herman J, Schulick R, Hruban RH and Goggins M. Pancreatic cancer. Lancet 2011; 378: 607-620.

[3] Sohn TA, Yeo CJ, Cameron JL, Koniaris L, Kaushal S, Abrams RA, Sauter PK, Coleman J, Hruban RH and Lillemoe KD. Resected adenocarcinoma of the pancreas-616 patients: results, outcomes, and prognostic indicators. J Gastrointest Surg 2000; 4: 567-579.

[4] Yeo CJ, Abrams RA, Grochow LB, Sohn TA, Ord SE, Hruban RH, Zahurak ML, Dooley WC, Coleman J, Sauter PK, Pitt HA, Lillemoe KD and Cameron JL. Pancreaticoduodenectomy for pancreatic adenocarcinoma: postoperative adjuvant chemoradiation improves survival. A

prospective, single-institution experience. Ann Surg 1997; 225: 621-633; discussion 633-626.
[5] Westgaard A, Schjolberg AR, Cvancarova M, Eide TJ, Clausen OP and Gladhaug IP. Differentiation markers in pancreatic head adenocarcinomas: MUC1 and MUC4 expression indicates poor prognosis in pancreatobiliary differentiated tumours. Histopathology 2009; 54: 337-347.
[6] Yamasaki H, Ikeda S, Okajima M, Miura Y, Asahara T, Kohno N and Shimamoto F. Expression and localization of MUC1, MUC2, MUC5AC and small intestinal mucin antigen in pancreatic tumors. Int J Oncol 2004; 24: 107-113.
[7] Yonezawa S, Horinouchi M, Osako M, Kubo M, Takao S, Arimura Y, Nagata K, Tanaka S, Sakoda K, Aikou T and Sato E. Gene expression of gastric type mucin (MUC5AC) in pancreatic tumors: its relationship with the biological behavior of the tumor. Pathol Int 1999; 49: 45-54.
[8] Zhu Y, Zhang JJ, Zhu R, Zhu Y, Liang WB, Gao WT, Yu JB, Xu ZK and Miao Y. The increase in the expression and hypomethylation of MUC4 gene with the progression of pancreatic ductal adenocarcinoma. Med Oncol 2011; 28 Suppl 1: S175-S184.
[9] Argani P, Iacobuzio-Donahue C, Ryu B, Rosty C, Goggins M, Wilentz RE, Murugesan SR, Leach SD, Jaffee E, Yeo CJ, Cameron JL, Kern SE and Hruban RH. Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE). Clin Cancer Res 2001; 7: 3862-3868.
[10] Baruch AC, Wang H, Staerkel GA, Evans DB, Hwang RF and Krishnamurthy S. Immunocytochemical study of the expression of mesothelin in fine-needle aspiration biopsy specimens of pancreatic adenocarcinoma. Diagn Cytopathol 2007; 35: 143-147.
[11] Bhardwaj A, Marsh WL Jr, Nash JW, Barbacioru CC, Jones S and Frankel WL. Double immunohistochemical staining with MUC4/p53 is useful in the distinction of pancreatic adenocarcinoma from chronic pancreatitis: a tissue microarray-based study. Arch Pathol Lab Med 2007; 131: 556-562.
[12] Chu PG, Schwarz RE, Lau SK, Yen Y and Weiss LM. Immunohistochemical staining in the diagnosis of pancreatobiliary and ampulla of Vater adenocarcinoma: application of CDX2, CK17, MUC1, and MUC2. Am J Surg Pathol 2005; 29: 359-367.
[13] Hassan R, Laszik ZG, Lerner M, Raffeld M, Postier R and Brackett D. Mesothelin is overexpressed in pancreaticobiliary adenocarcinomas but not in normal pancreas and chronic pancreatitis. Am J Clin Pathol 2005; 124: 838-845.
[14] Jinfeng M, Kimura W, Hirai I, Sakurai F, Moriya T and Mizutani M. Expression of MUC5AC and MUC6 in invasive ductal carcinoma of the pancreas and relationship with prognosis. Int J Gastrointest Cancer 2003; 34: 9-18.
[15] Lau SK, Weiss LM and Chu PG. Differential expression of MUC1, MUC2, and MUC5AC in carcinomas of various sites: an immunohistochemical study. Am J Clin Pathol 2004; 122: 61-69.
[16] McCarthy DM, Maitra A, Argani P, Rader AE, Faigel DO, Van Heek NT, Hruban RH and Wilentz RE. Novel markers of pancreatic adenocarcinoma in fine-needle aspiration: mesothelin and prostate stem cell antigen labeling increases accuracy in cytologically borderline cases. Appl Immunohistochem Mol Morphol 2003; 11: 238-243.
[17] Swartz MJ, Batra SK, Varshney GC, Hollingsworth MA, Yeo CJ, Cameron JL, Wilentz RE, Hruban RH and Argani P. MUC4 expression increases progressively in pancreatic intraepithelial neoplasia. Am J Clin Pathol 2002; 117: 791-796.
[18] Takikita M, Altekruse S, Lynch CF, Goodman MT, Hernandez BY, Green M, Cozen W, Cockburn M, Sibug Saber M, Topor M, Zeruto C, Abedi-Ardekani B, Reichman ME and Hewitt SM. Associations between selected biomarkers and prognosis in a population-based pancreatic cancer tissue microarray. Cancer Res 2009; 69: 2950-2955.
[19] Hoshi H, Sawada T, Uchida M, Saito H, Iijima H, Toda-Agetsuma M, Wada T, Yamazoe S, Tanaka H, Kimura K, Kakehashi A, Wei M, Hirakawa K and Wanibuchi H. Tumor-associated MUC5AC stimulates in vivo tumorigenicity of human pancreatic cancer. Int J Oncol 2011; 38: 619-627.
[20] Gold DV, Karanjawala Z, Modrak DE, Goldenberg DM and Hruban RH. PAM4-reactive MUC1 is a biomarker for early pancreatic adenocarcinoma. Clin Cancer Res 2007; 13: 7380-7387.
[21] Gold DV, Lew K, Maliniak R, Hernandez M and Cardillo T. Characterization of monoclonal antibody PAM4 reactive with a pancreatic cancer mucin. Int J Cancer 1994; 57: 204-210.
[22] Iacobuzio-Donahue CA, Fu B, Yachida S, Luo M, Abe H, Henderson CM, Vilardell F, Wang Z, Keller JW, Banerjee P, Herman JM, Cameron JL, Yeo CJ, Halushka MK, Eshleman JR, Raben M, Klein AP, Hruban RH, Hidalgo M and Laheru D. DPC4 gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. J Clin Oncol 2009; 27: 1806-1813.

[23] Iacobuzio-Donahue CA, Wilentz RE, Argani P, Yeo CJ, Cameron JL, Kern SE and Hruban RH. Dpc4 protein in mucinous cystic neoplasms of the pancreas: frequent loss of expression in invasive carcinomas suggests a role in genetic progression. Am J Surg Pathol 2000; 24: 1544-1548.

[24] Hruban RH, Goggins M and Kern SE. Molecular genetics and related developments in pancreatic cancer. Curr Opin Gastroenterol 1999; 15: 404-409.

[25] Liu H, Shi J, Anandan V, Wang HL, Diehl D, Blansfield J, Gerhard G and Lin F. Reevaluation and identification of the best immunohistochemical panel (pVHL, Maspin, S100P, IMP-3) for ductal adenocarcinoma of the pancreas. Arch Pathol Lab Med 2012; 136: 601-609.

[26] Park SY, Kim BH, Kim JH, Lee S and Kang GH. Panels of immunohistochemical markers help determine primary sites of metastatic adenocarcinoma. Arch Pathol Lab Med 2007; 131: 1561-1567.

[27] Yeh TS, Ho YP, Chiu CT, Chen TC, Jan YY and Chen MF. Aberrant expression of cdx2 homeobox gene in intraductal papillary-mucinous neoplasm of the pancreas but not in pancreatic ductal adenocarcinoma. Pancreas 2005; 30: 233-238.

[28] Saitou M, Goto M, Horinouchi M, Tamada S, Nagata K, Hamada T, Osako M, Takao S, Batra SK, Aikou T, Imai K and Yonezawa S. MUC4 expression is a novel prognostic factor in patients with invasive ductal carcinoma of the pancreas. J Clin Pathol 2005; 58: 845-852.

[29] Tascilar M, Skinner HG, Rosty C, Sohn T, Wilentz RE, Offerhaus GJ, Adsay V, Abrams RA, Cameron JL, Kern SE, Yeo CJ, Hruban RH and Goggins M. The SMAD4 protein and prognosis of pancreatic ductal adenocarcinoma. Clin Cancer Res 2001; 7: 4115-4121.

[30] Hassan R, Bera T and Pastan I. Mesothelin: a new target for immunotherapy. Clin Cancer Res 2004; 10: 3937-3942.

[31] Hassan R, Bullock S, Premkumar A, Kreitman RJ, Kindler H, Willingham MC and Pastan I. Phase I study of SS1P, a recombinant anti-mesothelin immunotoxin given as a bolus I.V. infusion to patients with mesothelin-expressing mesothelioma, ovarian, and pancreatic cancers. Clin Cancer Res 2007; 13: 5144-5149.

[32] Hassan R, Cohen SJ, Phillips M, Pastan I, Sharon E, Kelly RJ, Schweizer C, Weil S and Laheru D. Phase I clinical trial of the chimeric anti-mesothelin monoclonal antibody MORAb-009 in patients with mesothelin-expressing cancers. Clin Cancer Res 2010; 16: 6132-6138.

[33] Hassan R and Ho M. Mesothelin targeted cancer immunotherapy. Eur J Cancer 2008; 44: 46-53.

[34] Hassan R, Viner JL, Wang QC, Margulies I, Kreitman RJ and Pastan I. Anti-tumor activity of K1-LysPE38QQR, an immunotoxin targeting mesothelin, a cell-surface antigen overexpressed in ovarian cancer and malignant mesothelioma. J Immunother 2000; 23: 473-479.

[35] Hisatsune A, Nakayama H, Kawasaki M, Horie I, Miyata T, Isohama Y, Kim KC and Katsuki H. Anti-MUC1 antibody inhibits EGF receptor signaling in cancer cells. Biochem Biophys Res Commun 2011; 405: 377-381.

[36] Li M, Bharadwaj U, Zhang R, Zhang S, Mu H, Fisher WE, Brunicardi FC, Chen C and Yao Q. Mesothelin is a malignant factor and therapeutic vaccine target for pancreatic cancer. Mol Cancer Ther 2008; 7: 286-296.

[37] Karacay H, Sharkey RM, Gold DV, Ragland DR, McBride WJ, Rossi EA, Chang CH and Goldenberg DM. Pretargeted radioimmunotherapy of pancreatic cancer xenografts: TF10-90Y-IMP-288 alone and combined with gemcitabine. J Nucl Med 2009; 50: 2008-2016.

[38] Gold DV, Cardillo T, Vardi Y and Blumenthal R. Radioimmunotherapy of experimental pancreatic cancer with 131I-labeled monoclonal antibody PAM4. Int J Cancer 1997; 71: 660-667.

[39] Gold DV, Schutsky K, Modrak D and Cardillo TM. Low-dose radioimmunotherapy ((90)Y-PAM4) combined with gemcitabine for the treatment of experimental pancreatic cancer. Clin Cancer Res 2003; 9: 3929S-3937S.

[40] Mimeault M, Johansson SL, Senapati S, Momi N, Chakraborty S and Batra SK. MUC4 down-regulation reverses chemoresistance of pancreatic cancer stem/progenitor cells and their progenies. Cancer Lett 2010; 295: 69-84.

[41] Inaguma S, Kasai K and Ikeda H. GLI1 facilitates the migration and invasion of pancreatic cancer cells through MUC5AC-mediated attenuation of E-cadherin. Oncogene 2011; 30: 714-723.

[42] Maitra A, Adsay NV, Argani P, Iacobuzio-Donahue C, De Marzo A, Cameron JL, Yeo CJ and Hruban RH. Multicomponent analysis of the pancreatic adenocarcinoma progression model using a pancreatic intraepithelial neoplasia tissue microarray. Mod Pathol 2003; 16: 902-912.

[43] Hollingsworth MA and Swanson BJ. Mucins in cancer: protection and control of the cell surface. Nat Rev Cancer 2004; 4: 45-60.

[44] Swanson BJ, McDermott KM, Singh PK, Eggers JP, Crocker PR and Hollingsworth MA. MUC1 is a counter-receptor for myelin-associated glycoprotein (Siglec-4a) and their interaction contributes to adhesion in pancreatic cancer perineural invasion. Cancer Res 2007; 67: 10222-10229.

[45] Singh PK and Hollingsworth MA. Cell surface-associated mucins in signal transduction. Trends Cell Biol 2006; 16: 467-476.
[46] Argani P, Shaukat A, Kaushal M, Wilentz RE, Su GH, Sohn TA, Yeo CJ, Cameron JL, Kern SE and Hruban RH. Differing rates of loss of DPC4 expression and of p53 overexpression among carcinomas of the proximal and distal bile ducts. Cancer 2001; 91: 1332-1341.