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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

RESEARCH LETTER

Diagnosis of Malignant Potential in Mucinous Peritoneal Neoplasms by Characterization of Mucin Carbohydrate Structure

Pseudomyxoma peritonei (PMP) is characterized by the growth of intestinal epithelial cells with extensive mucin secretion in the abdomen and pelvis. The cells grow freely in the peritoneal cavity and may be a low-grade mucinous neoplasm or a peritoneal mucinous carcinoma. However, the mucinous nature and the peritoneal location make it difficult to determine whether the neoplastic epithelial cells are benign or malignant. Consequently, it is difficult to determine how the tumor will behave, predict the long-term outcome, or plan rational therapy.

It has long been appreciated that mucins in the normal colon have a homogeneous pattern of terminal glycosylation that is altered in the mucins associated with colorectal cancer (CRC), which can be recognized by lectin binding patterns.[1] The goal of this study was to use lectin binding patterns in the setting of PMP to characterize mucins secreted by low-grade neoplasms and those produced by adenocarcinomas using fluorescein isothiocyanate (FITC)-labeled lectins: FITC–*Dolichos biflorus* agglutinin (DBA), FITC–soybean agglutinin, FITC–*Ricinus communis* agglutinin-1, FITC–*Ulex europeus* agglutinin-1, and FITC–peanut agglutinin (PNA). We hypothesized that there would be differences in glycoconjugate structure that would differentiate mucins in adenocarcinomas from those in lower grade neoplasms.

The PMP cases were divided into 2 pathologic groups (low-grade benign mucinous neoplasms and mucinous adenocarcinomas), and the lectin binding was independently determined to be positive or negative for each case. Forty-five patients were studied; 25 had adenocarcinoma, and 20 a low-grade neoplasm (Table 1).

All of the adenocarcinomas were labeled by FITC-PNA, versus 50% of the low-grade neoplasms ($P < .01$) (Table 2, Supplementary Figures 1–4). The other lectin-binding results are in Table 2.

Mucus is the term for the viscoelastic substance coating the intestinal epithelium, and its principal nonaqueous component is mucin. Mucin apoproteins have a linear polypeptide chain in its central axis.[2] The oligosaccharide side chains of mucins often terminate with sialic acids or sulfate groups, making them negatively charged and hydrophilic. It has been demonstrated that virtually all normal human colonic mucins have the same terminal sugars on their oligosaccharide side chains.[1] Goblet cells in the upper portion of the colonic crypt synthesize mucin with a terminal sugar that is bound by the lectins DBA and soybean agglutinin (α-*N*-acetylgalactosamine). There is a gradient of labeling by DBA and soybean agglutinin as goblet cells differentiate and migrate up the colonic crypt. The goblet cell mucins at the bottom of the crypt terminate with a sugar recognized by *Ricinus communis* agglutinin-1 (β-galactose). The disappearance of the terminal β-linked Gal residues at the base of the crypt is perfectly matched by the appearance of terminal α-linked GalNAc residues at the top. However, CRCs lose the organized crypt structure and there is a change in the pattern of lectin binding. PNA binds to the mucins made by almost all CRCs but essentially never binds to the mucins made in normal colons.[3] PNA binds to the Thomsen-Friedenreich antigen (T-Ag), a cancer-associated sequence that consists of a short disaccharide pair (Galβ1-3->GalNAc).[4] In some instances, the Gal, GalNAc of T-Ag, or both may be extended by terminal α-linked sialic acid residues; PNA also binds sialylated T antigens.[4] Importantly, the presence of the PNA-binding glycoproteins can also be seen within large benign adenomatous polyps in foci of high-grade dysplasia.[3]

In this study, all of the mucinous adenocarcinomas were PNA-positive, indicating a cancer-associated T-Ag, whereas only half of low-grade neoplasms were PNA-positive. The other lectins were not as helpful diagnostically but shed light on the likely oligosaccharide structures in PMP (Table 2). One possibility is that terminal sialic acid residues are permissive of PNA-binding but inhibit *Ricinus communis* agglutinin-1 binding. DBA binds the terminal α-GalNAc of blood group A and labeling was present in about half of the adenocarcinomas but most of the low-grade neoplasms. It is likely that DBA recognizes different GalNAc residues in the cancers than in the normal colon, because in addition to recognizing the

Table 1. Demographics

	All	Type of neoplasm		*P* value
		Adenocarcinoma	Low-grade	
Age, *y*, mean ± SD	56.2 ± 16.1	56.3 ± 13.4	56.0 ± 19.4	.94[a]
Male gender, n (%)	19 (42.2)	11 (44.0)	8 (40.0)	1.00[b]
Race, n (%)				.31[c]
White	36 (80.0)	18 (72.0)	18 (90.0)	
African American	6 (13.3)	5 (20.0)	1 (5.0)	
Other, non-Hispanic	2 (4.4)	1 (4.0)	1 (5.0)	
Unknown	1 (2.2)	1 (4.0)	0 (0.0)	
Location				
Colon/rectum	13 (28.8)	13 (52.0)	0 (0.0)	
Appendix	26 (57.7)	6 (24.0)	20 (100.0)	
Unknown	6 (13.3)	6 (24.0)	0 (0.0)	

[a]*P* value calculated using Student *t* test.
[b]*P* value calculated using Fisher exact test.
[c]*P* value calculated using chi-square test.

Table 2. Summary of Lectin Binding Characteristics Using 5 FITC-Lectins

	All	Type of neoplasm		*P* value[a]
		Adenocarcinoma	Low-grade	
PNA				<.01
Positive	35 (77.8)	25 (100.0)	10 (50.0)	
Negative	10 (22.2)	0 (0.0)	10 (50.0)	
RCA-1				.03
Positive	22 (48.9)	8 (32.0)	14 (70.0)	
Negative	22 (48.9)	16 (64.0)	6 (30.0)	
Unknown	1 (2.2)	1 (4.0)	0 (0.0)	
SBA				1.00
Positive	18 (40.0)	10 (40.0)	8 (40.0)	
Negative	27 (60.0)	15 (60.0)	12 (60.0)	
UEA-1				.18
Positive	33 (73.3)	16 (64.0)	17 (85.0)	
Negative	12 (26.7)	9 (36.0)	3 (15.0)	
DBA				.01
Positive	29 (64.4)	12 (48.0)	17 (85.0)	
Negative	16 (35.6)	13 (52.0)	3 (15.0)	

DBA, *Dolichos biflorus* agglutinin; FITC, fluorescein isothiocyanate; PNA, peanut agglutinin; RCA-1, *Ricinus communis* agglutinin-1; SBA, soybean agglutinin; UEA-1, *Ulex europeus* agglutinin-1.
[a]*P* values calculated using Fisher exact test.

blood group A structure, DBA also binds to solitary α-linked GalNAc, such as that in the cancer-associated structure, Tn.[5] The presence of Tn and sialylated-Tn in mucins is associated with metastasis and a poorer prognosis.[6]

It has been previously shown that CRC-associated mucins are less densely glycosylated, have shorter oligosaccharide side chains than in the normal colon,[7] and have other biochemical differences in molecular weight and charge.[8] Future directions include purification and biochemical characterization of these cancer-associated mucins, and exploration of the molecular and enzymatic basis of this phenomenon.

L. AUGUST CLARK,[1] *ALEXIA GHAZI,*[2] *KRISTIN GAFFNEY,*[1] *RODRIGO SOTO,*[2] *ATIN AGARWAL,*[2] *SUSANNE CARMACK,*[2] *C. RICHARD BOLAND,*[1,3,4]

[1]Division of Gastroenterology, Department of Internal Medicine, Baylor University Medical Center, Baylor Scott & White Health Care, Dallas, Texas

[2]Department of Pathology, Baylor University Medical Center, Baylor Scott & White Health Care, Dallas, Texas

[3]Charles Sammons Cancer Center and Baylor Research Institute, Baylor University Medical Center, Baylor Scott & White Health Care, Dallas, Texas

[4]Current address: University of California San Diego, San Diego, California

Corresponding author: C. Richard Boland, MD, University of California San Diego, Medicine, GI Division, Gilman Drive, San Diego, California 92093. e-mail: crboland@ucsd.edu.

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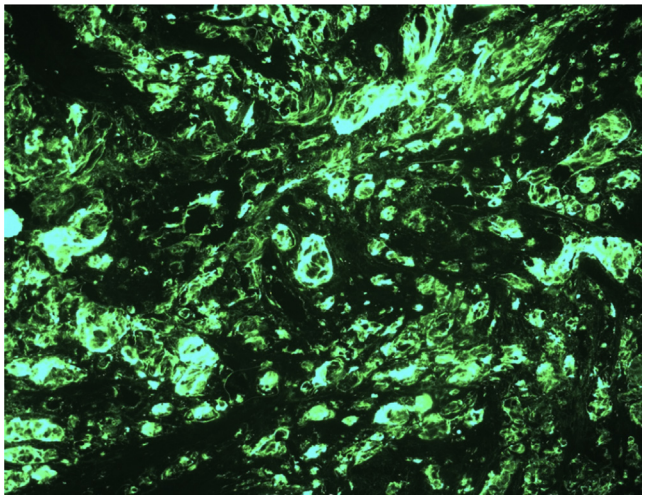
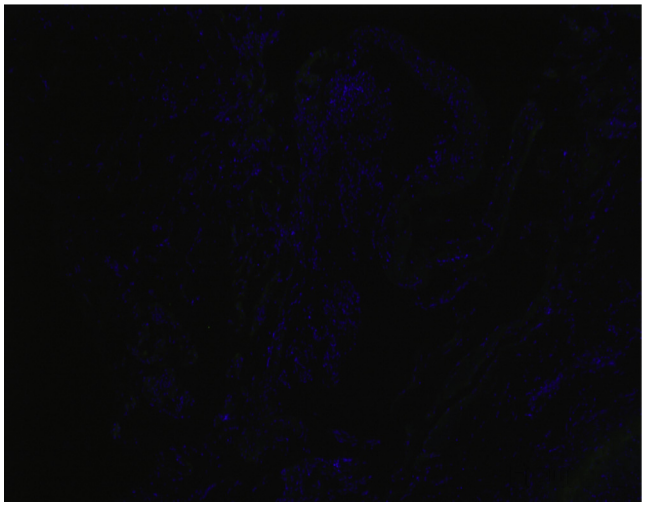
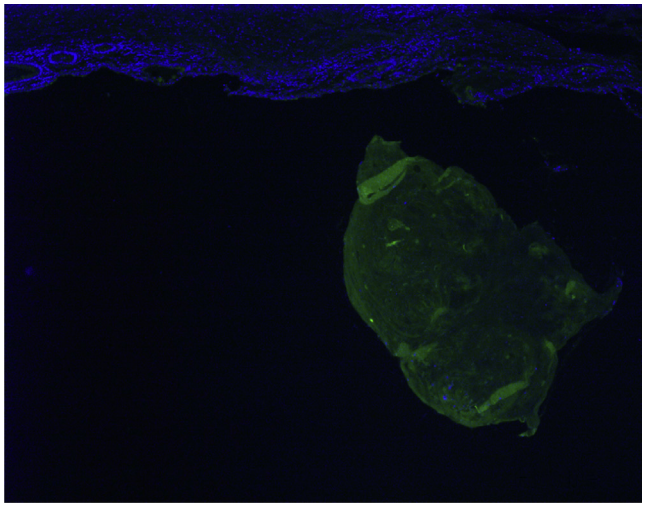
Conflicts of interest
The authors disclose no conflicts.



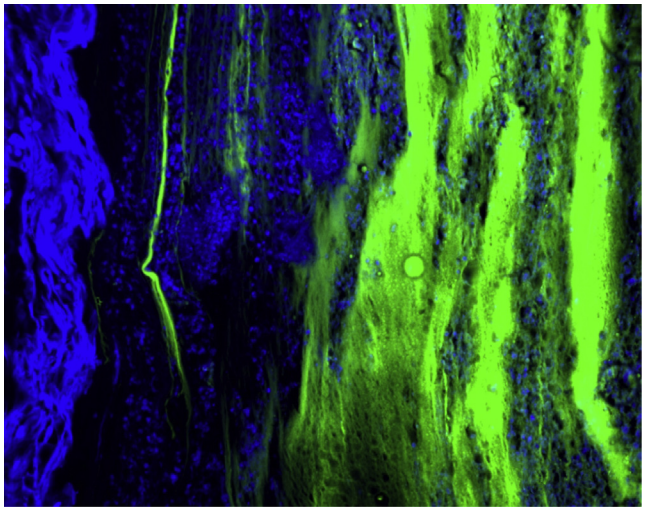
Supplementary Figure 1. Strongly positive peanut agglutinin staining within mucin deposition of an invasive mucinous adenocarcinoma (×10 objective, Zeiss epifluorescent microscope). The patient was later discharged to hospice.



Supplementary Figure 2. Negative peanut agglutinin staining within mucin deposition of a low-grade neoplasm (×10 objective Zeiss epifluorescent microscope). The 4′,6-diamidino-2-phenylindole counterstain highlights intact cell nuclei within the mucin.



Supplementary Figure 3. Positive *Ricinus communis* agglutinin-1 staining within mucin deposition of a low-grade neoplasm (×10 objective, Zeiss epifluorescent microscope). The 4′,6-diamidino-2-phenylindole counterstain highlights intact cell nuclei within the mucin and background benign colonic epithelium.



Supplementary Figure 4. Positive *Dolichos biflorus* agglutinin staining within mucin deposition of a low-grade neoplasm (×20 objective, Zeiss epifluorescent microscope). The 4′,6-diamidino-2-phenylindole counterstain highlights intact cell nuclei within the mucin and background benign colonic epithelium.