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Original Article

Smoothelin and caldesmon are reliable markers for distinguishing muscularis propria from desmoplasia: a critical distinction for accurate staging colorectal adenocarcinoma

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Abstract: An accurate distinction between deep muscularis propria invasion versus subserosal invasion by colonic adenocarcinoma is essential for the accurate staging of cancer and subsequent optimal patient management. However, problems may arise in pathologic staging when extensive desmoplasia blurs the junction between deep muscularis propria and subserosal fibroadipose tissue. To address this issue, forty-three (43) cases of colonic adenocarcinoma resections from 2007-2009 at The Methodist Hospital in Houston, TX were reviewed. These cases were selected to address possible challenges in differentiating deep muscularis propria invasion from superficial subserosal invasion based on H&E staining alone. Immunohistochemical staining using smooth muscle actin (SMA), smoothelin, and caldesmon were performed on 51 cases: 8 cases of pT1 tumors (used mainly as control); 12 pT2 tumors; and 31 pT3 tumors. All 51 (100%) had diffuse, strong (3+) immunoreactivity for caldesmon and smoothelin in the muscularis propria with a granular cytoplasmic staining pattern. However, the desmoplastic areas of these tumors, composed of spindled fibroblasts and myofibroblasts, showed negative immunostaining for caldesmon and smoothelin (0/35). SMA strongly stained the muscularis propria and weakly (1+) or moderately (2+) stained the spindled fibroblasts in the desmoplastic areas (the latter presumably because of myofibroblastic differentiation). Compared to SMA, caldesmon and smoothelin are more specific stains that allow better delineation of the muscularis propria from the desmoplastic stromal reaction which provides a critical aide for proper staging of colonic adenocarcinoma and subsequent patient care.

Keywords: Colon adenocarcinoma, immunohistochemistry, cancer staging

Introduction

Malignant neoplasms of the lower gastrointestinal tract are extremely common with 50,830 estimated deaths from colon and rectal cancers in 2012 [1]. Accurate staging of these neoplasms is of critical importance because diagnoses influence patient management and may ultimately determine patient survival. 5-year survival rates differ significantly from 74-86% for pT2 lesions [invasion into muscularis propria (MP)] versus 39-66% for pT3 disease (invasion into subserosal adipose tissue) [2, 3]. When intense desmoplastic response of the stroma obscures normal histologic landmarks, the assessment of depth of invasion of colonic adenocarcinoma is often challenging. The distinction between the robust desmoplastic response present at the junction of the MP and the subserosal fibroadipose tissue is critical to accurately stage invasive colorectal adenocarcinomas for appropriate patient management. Ancillary techniques including immunohistochemistry can be of particular assistance in this endeavor.

Smoothelin is a relatively novel cytoskeletal protein that is expressed in fully differentiated smooth muscle tissue; it is not present cells with smooth muscle-like contractile function, including myofibroblasts, myoepithelial cells and non-contractile smooth muscle cells [4, 5]. Prior studies applied smoothelin to urinary bladder cases and demonstrated its superior differ-

ential staining of MP (strong, diffuse) versus muscularis mucosa [(MM), weak to absent] [6, 7]. These findings are corroborated in the colon where similar findings of weaker to absent smoothelin staining of the MM as compared to MP [8]. Smoothelin has also been utilized for differentiating muscle layers in the upper gastrointestinal tract, including gallbladder, cystic duct, and common bile duct [9].

Additionally, caldesmon has been shown to be a reliable marker for differentiating reactive myofibroblasts (negative staining) found in desmoplastic stromal reactions from MP in invasive urothelial carcinoma cases [10]. Earlier studies established caldesmon as a negative marker of myofibroblasts, making it an attractive marker to differentiate desmoplasia and smooth muscle [11, 12].

The purpose of this study was to investigate the role of caldesmon, smoothelin, and smooth muscle actin (SMA) in distinguishing MP from desmoplastic fibroblastic/myofibroblastic proliferation in invasive colonic adenocarcinoma, supporting the valuable role of immunohistochemistry in proper staging of colon cancer, which leads to subsequent optimal patient care.

Materials and methods

Fifty-one segmental colectomy cases resected for colonic adenocarcinoma at Houston Methodist Hospital in Houston, TX during 2007-2009 were retrieved from archives. Twelve T2 tumors, and 31 T3 tumor cases were selected for this study, and eight T1 tumors were used as control. All cases were reviewed histologically and were specifically selected due to the challenge of differentiating deep MP invasion from superficial subserosal invasion (T2 and T3 tumors), and submucosal invasion alone (T1 tumors) from superficial MP invasion (T2 tumors) based on H&E staining alone.

Immunohistochemistry was performed on 5 μm thick formalin fixed, paraffin-embedded tissue. The following antibodies were used: caldesmon (h-CD; 1:50 dilution; Dako North America, Carpentaria, CA), SMA (1A4; prediluted; Ventana Medical Systems, Tucson, AZ), and smoothelin (R4A; 1:100 dilution; Abcam Inc, Cambridge, MA). Tissue sections were incubated with the primary antibody for a total of thirty minutes at room temperature, subsequently washed with phosphate buffered saline, and then incubated with a secondary antibody that was conjugated to horseradish peroxidase (Benchmark IHC/ISH module; Ventana, Tucson, AZ). Interpretation of immunoreactivity of the antibodies was analyzed in a semiquantitative manner by interpreting the staining positivity of smooth muscle cells and fibroblasts/myofibroblasts. Staining was evaluated as follows: negative = less than or equal to 5% positivity, weak (1+) = 6-20% positivity, moderate (2+) = 21-50%, and strong (3+) = 51-100% cell positivity. Sections of normal colonic proper muscle wall were used for positive control and omission of the primary antibodies was used as the negative control in all cases.

Results

Clinical findings

Of the 51 patients selected for this study, 29 were male (57%) and 22 were female (43%). The median age of the patients was 69 years old (range 34-91 years; mean = 67.9 years). The sites of colonic adenocarcinomas were as follows: 18 cases were in the right colon (35.3%), 12 in the sigmoid colon (23.5%), 10 in the rectum (20%), 5 in the left colon (9.8%), 4 in the transverse colon (7.8%), and 2 in a site not designated (3.9%).

H&E light microscopic findings

In total, 43 cases of deep muscle invasive colonic adenocarcinoma (12 pT2 tumors, and 31 pT3 tumors) and 8 pT1 tumors were reviewed. Desmoplastic stromal response to invasive carcinoma, characterized by a cellular proliferation of fibroblasts and myofibroblasts surrounding tumors, was more commonly seen in more deeply invasive carcinomas and identified in 31 cases including 26 pT3 (84% of pT3 tumors), 5 pT2 (42% of pT2 tumors) and 4 pT1 (50% of pT1 tumors) cancers.

Immunohistochemical findings

All 51 cases (100%) had diffuse and strong (3+) immunoreactivity for SMA, caldesmon, smoothelin in the muscularis propria with a granular cytoplasmic staining pattern. Of the 43 cases of deep muscle invasive colonic adenocarcinomas, 31 (72.1%) showed a desmoplastic stro-

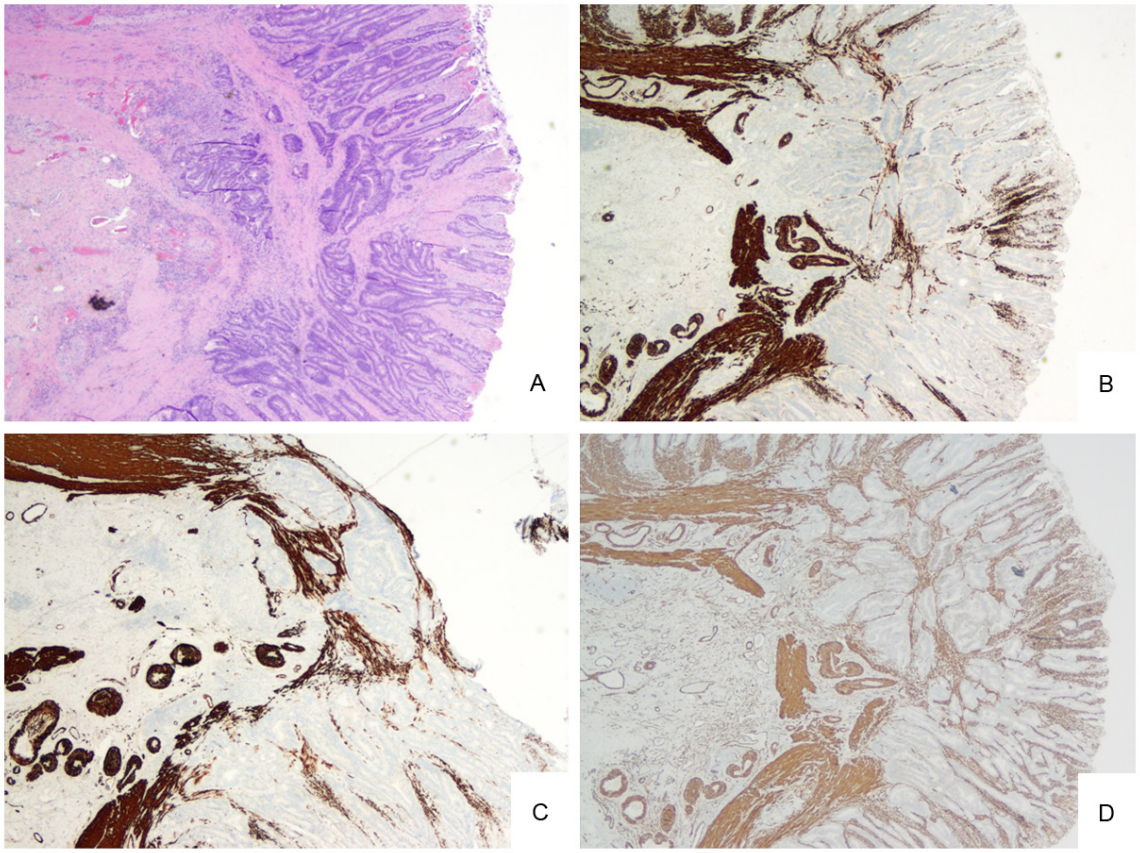

Figure 1. Sections demonstrate colonic adenocarcinoma with significant desmoplasia of peri-intestinal adipose tissue and distortion of the junction between muscularis propria (MP) and subserosa (A). Immunostaining with Caldesmon (B) and Smoothelin (C) help differentiate MP from desmoplastic response while smooth muscle actin does not (D).

mal response to invasive carcinoma, and of those cases twenty-six were pT3 (84%) and 5 were pT2 (16%). Desmoplastic reaction was seen in 4/8 pT1 tumors (50%). The spindled fibroblasts and myofibroblasts found in the desmoplastic areas of all of these tumors showed negative immunostaining for both caldesmon and smoothelin (0/35). However, while SMA strongly (3+) stained the MP, it also weakly (1+) or moderately (2+) stained the spindled fibroblasts/myofibroblasts in the desmoplastic stroma (**Figure 1**). It was also noted in all cases that the MM was found to have moderate to strong staining for SMA, but weak (1+) or negative cytoplasmic staining with smoothelin and caldesmon.

Discussion

The staging of invasive colonic adenocarcinoma can sometimes present challenges to the surgical pathologists when intense desmoplastic stromal response in routine H&E stained tissue sections makes the distinction between MP and subserosal adipose tissue with fibroblastic/myofibroblastic reaction difficult. This is important because accurate cancer staging is critically dependent on proper recognition of the depth of tumor invasion into the anatomic layers of the colonic wall. Furthermore, the stage of colon cancer portends significantly different patient survival rates and postoperative adjuvant chemotherapy selection [2, 3].

Previous studies of immunohistochemistry as a tool to assess depth of invasion of carcinoma to assist in staging has involved the urinary bladder where the differential staining pattern of smoothelin has proven to be a specific and useful marker to determine MM versus MP invasion [6, 10]. This is a critical distinction in the urinary bladder because when MP invasion is identified (pT2), patients typically receive aggressive treatment which may include cys-

tectomy, chemotherapy and radiation, significantly different from intravesical chemotherapy or BCG treatment in MM invading pT1 tumors. Similarly, colonic adenocarcinoma staging is determined in great part by invasion of the MP (pT2) or subserosal adipose tissue (pT3), which also affects the prognosis and subsequent management for the patient [13].

Miyamoto et al. reaffirmed the differential staining of smoothelin in MM and MP in transurethral resections of urinary bladder but cautioned the interpretation due to occasional overlap in staining patterns [7]. Smoothelin has been shown to be a useful addition to the diagnostic armamentarium of the surgical pathologist in the urinary bladder. Challenges arise when reactive fibroblasts/myofibroblasts in desmoplastic stromal response to invasive carcinoma mimic smooth muscle bundles. Caldesmon paired with smoothelin are more specific than SMA in highlighting MM- type smooth muscle fibers versus MP-type smooth muscle fibers and smooth muscle cells versus reactive fibroblasts/myofibroblasts in desmoplastic stroma in cystectomy specimens [10].

Our current study demonstrates that smoothelin is a specific marker to differentiate MM from desmoplasic stromal response to invasive tumor and therefore has implications for staging of gastrointestinal tract adenocarcinomas. Also, smoothelin has been shown to be particularly useful for small biopsy specimens and endoscopic mucosal resections where intense desmoplasia, repetitive endoscopic mucosal resection or neoadjuvant treatment can distort normal anatomic landmarks [8]. In addition, smoothelin can be used to distinguish between duplicated MM seen in Barrett esophagus for accurate staging of superficial invasive carcinomas found in biopsy specimens and endoscopic mucosal resections [14]. In these situations, adjunct immunohistochemical stains are extremely useful for the surgical pathologist. Smoothelin has also been noticed to be consistently negative in reactive desmoplastic areas adjacent to invasive carcinoma throughout the gastrointestinal tract as compared to SMA which showed scattered to intense positive staining [7]. Despite these prior studies, there is lack of data regarding utilization of immunohistochemistry as an ancillary tool to assist in distinction from desmoplastic response to invasive carcinoma and true tumor invasion into the MP. Furthermore, caldesmon has not been thoroughly investigated as a distinguishing marker in this regard despite having negative staining in fibroblasts/myofibroblasts [11, 12].

Preceding studies have shown caldesmon and smoothelin to be reliable in distinguishing between true MP and desmoplastic response to urothelial invasive carcinoma. However, no study addressed application of these stains in the colon, especially in regard to differentiating deep MP invasion from pericolonic fibroadipose invasion with desmoplastic reaction. Based upon our findings, caldesmon and smoothelin have great value in making this critical distinction. Caldesmon and smoothelin both strongly stained the MP in all cases of deep muscle invasive colonic adenocarcinoma (43/43) as well as 8 T1 tumors with submucosal invasion and did not stain the desmoplastic fibroblast/myofibroblast population when present. SMA is not a discriminatory stain in this setting, because weak to moderate staining was seen in most cases of the reactive fibroblast/myofibroblast population in addition to staining in MP.

Since the clinical implications in staging and the difficulty in making an accurate assessment of depth of invasion due to frequent distortion of the junction between MP and pericolonic fibroadipose tissue by intense desmoplastic stromal response to invasive tumor, caldesmon and smoothelin provide a useful ancillary tool for the surgical pathologist.

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Disclosure of conflict of interest

None.

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