Pathology – Research and Practice 212 (2016) 545–548

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original article

Overexpression of α1 chain of type XI collagen (COL11A1) aids in the diagnosis of invasive carcinoma in endoscopically removed malignant colorectal polyps

Dongwei Zhang, Hongfa Zhu, Noam Harpaz*

Department of Pathology, Icahn School of Medicine at Mount Sinai, NY, NY, USA

ARTICLE INFO

Article history:
Received 19 August 2015
Received in revised form 9 March 2016
Accepted 15 March 2016

Keywords:
Malignant colorectal polyps
Invasive adenocarcinoma
Misplaced adenomatous mucosa
Desmoplastic stroma
α1 chain of type XI collagen (COL11A1)

ABSTRACT

Introduction: The histologic distinction between benign misplaced adenomatous mucosa and invasive adenocarcinoma in colorectal polyps can be challenging. The α1 chain of type XI collagen (COL11A1) has been shown to be overexpressed in cancer-associated stromal fibroblasts. The aim of this study was to investigate the operating characteristics of COL11A1 immunostaining in benign and malignant colorectal polyps as a potential diagnostic aid.
Materials and methods: Sixty-six endoscopically-removed paraffin-embedded colorectal polyps were stained with anti-COL11A1 antibody. They comprised 50 malignant polyps (MPs) with invasive adenocarcinoma (including 5 with concurrent benign misplaced adenomatous mucosa) and 16 adenomas, 11 with and 5 without benign misplaced adenomatous mucosa.
Results: COL11A1 was expressed either diffusely or focally in cancer-associated desmoplastic fibroblasts in 72.0% (36/50) of MPs. The rates of COL11A1 expression and the immunohistochemical staining patterns of the COL11A1 were positively correlated with the depth of cancer invasion in MPs. COL11A1 was expressed in all 9 (100%) MPs with a mucinous component and in 16/18 (88.9%) MPs associated with significant electrocautery effects. COL11A1 was not expressed adjacent to conventional adenomas or in the stroma adjacent to misplaced adenomatous mucosa.
Conclusions: COL11A1 antibody can assist in distinguishing the cancer-associated desmoplastic stroma from that associated with misplaced adenomatous mucosa. It is particularly helpful when electrocautery artifacts or mucin pools interfere with the diagnosis of invasive carcinoma. However, COL11A1 has limited value in diagnosing superficially invasive carcinomas with very little desmoplastic stroma due to its low positive rate and focal expression in some cases.

1. Introduction

Misplaced adenomatous mucosa (MAM), or "pseudoinvasion," is a phenomenon frequently encountered in large adenomatous colonic polyps that results from traumatic herniation of surface tissue into the stalk or submucosa. Despite its superficial histological resemblance to submucosally invasive adenocarcinoma, MAM is usually recognized without difficulty on the basis of established morphological criteria, including a lobulated pattern of glandular aggregates, rounded profiles, cytological similarity between the submucosal and surface adenomatous crypts, incorporation of lamina propria into the herniated tissue, adjacent hemosiderin deposits and absence of a desmoplastic peritumoral reaction [1–3]. Nonetheless, one occasionally may encounter individual cases in which the distinction between MAM and invasive cancer is challenging, such as polyps containing large mucinous pools, distorted lobular pattern, mixed misplaced and invasive elements or electrocautery artifacts [4,5].

The use of adjunctive immunohistochemical markers to assist in the classification of diagnostically difficult cases has been described. Hansen et al. reported that expression of urokinase plasminogen activator receptor (uPAR) occurs in adenomas containing invasive carcinoma but rarely in those with misplaced mucosa [6]. The use of differential expression of other epithelial and mesenchymal markers including E-cadherin, matrix metalloproteinase-1 (MMP-1) and collagen IV has been less successful due to limited sensitivity and specificity [7,8]. Expression of the metalloproteinase

* Corresponding author at: Department of Pathology, The Icahn School of Medicine at Mount Sinai, 1468 Madison Avenue, New York, NY 10029, USA.
E-mail addresses: noam.harpaz@gmail.com, noam.harpaz@mountsinai.org (N. Harpaz).

http://dx.doi.org/10.1016/j.prp.2016.03.005
0344-0338/

stromelysin-3 (ST 3) occurred in early invasive carcinomas but was lost in expansive invasive neoplasms and diffusely infiltrative invasive tumors with a lymphocytic peritumoral reaction [9].

The desmoplastic reaction which usually accompanies submucosally invasive adenocarcinoma is associated with deposition of extracellular matrix (ECM), dysregulation of ECM turnover, accelerated proliferation of fibroblasts, degradation of normal type IV collagen-rich basement membrane and accumulation of interstitial fibrillar collagens [10,11]. α1 chain of type XI collagen (COL11A1) is a relatively minor fibrillar collagen that is an integral component of the cartilage fibrillar network in the normal tissues [12]. Its expression has been observed immunohistochemically in the stromal fibroblasts adjacent to colorectal, gastric, breast, pancreatic and ovarian cancers but not adjacent to benign epithelia, suggesting that it might be specific for cancer-associated ECM [13–16]. These studies were performed mostly on human cancer cell lines or on resection specimens, and occasionally on core biopsies. In this study we investigated the operating characteristics of COL11A1 expression in the ECM of invasive colorectal cancer and its potential diagnostic value in the classification of problematic colorectal polyps.

2. Materials and methods

Sixty-six colorectal endoscopic polypectomy specimens comprising 50 malignant polyps (MPs) and 16 benign adenomas were identified at random from our departmental electronic anatomic pathology database. The MPs comprised 16 polyps with superficially invasive adenocarcinoma involving upper third of the submucosa (sm1), 11 of which lacked and 5 of which contained submucosal MAM in addition to carcinoma, as well as 34 polyps with invasive adenocarcinoma involving at least the middle third of the submucosa (sm2 or deeper). Sixteen benign adenomas were selected as controls, 11 with and 5 without MAM. The total of polyps with MAM was therefore 16, 5 in MPs with sm1 invasion and 11 benign.

Formalin-fixed paraffin-embedded (FFPE) tissue blocks of the polyps were sectioned and stained with anti-COL11A1 antibody (Oncomatryx, Spain) according to the manufacturer's protocol. Following preheating at 65 °C, pH 9, epitope retrieval at 95 °C for 20 min with Dako PT link (Cat no. PT100/PT101, DAKO, Carpinteria, CA) and immersion for up to 5 min in diluted EnVision FLEX wash buffer (x10) (Cat No. K8000/K8010) at room temperature, the slides were incubated for 20 min with 100–200 μl of antibody at 1:100 dilution at room temperature. They were then incubated with Optiview HQ (Dako, CA) universal linker for 12 min and Optiview HRP multimer (Dako, CA) for 8 min.

The original hematoxylin-eosin-stained and the immunostained slides were reviewed independently and in blinded fashion by the 3 authors, 2 of whom are experienced gastrointestinal pathologists. The original pathologic diagnoses were confirmed in all cases.

COL11A1 expression in the stromal cells was cytoplasmic and produced intense, granular staining. The extent of staining in the individual polyps varied and was graded negative, focally positive (<10 cells/high power field) and diffusely positive (≥10 cells/high power field).

Comparison studies used Student's *t*-test, Chi-square test, and Fisher's exact test. The significance threshold level for the *P* value was less than 0.05.

3. Results

Pertinent demographics and polyp characteristics are summarized in Table 1. There were no statistically significant differences in age, gender, location and polyp size between the malignant polyp group and benign adenoma group. 15 of the 16 polyps with MAM were left-sided (93.8%).

Table 1
Demographics and clinical characteristics of colorectal polyps.

	Malignant polyps (n = 50)	Benign adenomas (n = 16)	*P* value
Age (years)	67.2 (40–89)	61.8 (40–85)	0.14
Gender			0.48
Male	23	9	
Female	27	7	
Location			0.18
Right colon	8	0	
Left colon	42	16	
Size (mm)	13.7 (6–25)	15.4 (8–22)	0.20

Table 2
Summary of COL11A1 immunohistochemistry in colorectal polyps.

Colorectal polyps	Positive COL11A1 reactivity (%)
Malignant polyps (n = 50)	36/50 (72.0%)
Benign glands (n = 21)	0/21 (0.0%)

*$P < 0.0001$.

As summarized in Table 2, expression of COL11A1 was observed in stromal cells adjacent to invasive cancer in 36 of the 50 MPs (72%). In contrast, expression of COL11A1 was not detected in 16 polyps with MAM and 5 benign adenomatous polyps without MAM (total 21). The positive predictive value of the staining is 100% and the negative predictive value is 60%.

The rates of COL11A1 expression and the immunohistochemical staining patterns of COL11A1 were positively correlated with the depth of cancer invasion in MPs. Thirty of 34 (88.2%) MPs with sm2 or deeper invasion were positive for COL11A1, whereas only 6 of 16 (37.5%) MPs with sm1 superficial invasion were positive for COL11A1. Additionally, the pattern of COL11A1 expression was diffuse in 19 (55.9%) and a focal pattern in 11 (32.4%) MPs with invasive adenocarcinoma of sm2 or deeper invasion. In contrast, COL11A1 exhibited a diffuse pattern in only 1 (6.3%) and a focal pattern in 5 (31.3%) MPs with sm1 invasive adenocarcinoma (Table 3).

No significant difference was detected between the COL11A1-positive and -negative cases with respect to tumor size (data not shown). Diffuse staining occurred in a variable proportion of the stromal cells and was readily seen at low (40×) magnification, whereas focal staining was identified under higher magnification. COL11A1 expression was detected neither in MAM (both in MPs and benign adenomas) nor in any of the benign polyps. Its expression in the MPs with submucosal MAM was limited to the stroma adjacent to the invasive malignant components but undetectable adjacent to the MAM (Fig. 1).

COL11A1 was expressed in all of 9 MPs (100%) that contained adenocarcinoma with a mucinous component. In contrast, COL11A1 expression was not detected in mucinous MAM (Fig. 2A and B). COL11A1 was likewise expressed in 16/18 (88.9%) MPs with significant electrocautery artifact and maintained its staining pattern compared to the non-cauterized cancer tissue in the same polyp. In contrast, COL11A1 expression was not detected in cauterized MAM (Table 4) (Fig. 2C and D).

4. Discussion

The interstitial extracellular matrix (ECM) plays crucial roles in cancer cell biology including morphogenesis, proliferation, tumor invasion, metastasis, and chemoresistance [17,18]. Expression of the ECM component COL11A1, the α1 chain of procollagen type