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Serrated epithelial colorectal polyps (hyperplastic polyps, sessile serrated adenomas) with perineurial stroma: Clinicopathological and molecular analysis of a new series

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ABSTRACT

Serrated colorectal fibroblastic polyps (FPs) are rare benign mucosal lesions composed of serrated epithelial crypts separated and distorted by intimately associated bland spindle cell proliferations with perineurial-like phenotype. We herein describe 21 new FPs affecting 10 females and 9 males aged 45 to 80 yrs. (mean, 62 yrs). Lesions originated in the sigmoid colon/rectosigmoid junction ($n = 16$), rectum ($n = 2$), and other parts of the colon ($n = 3$). Most patients had additional synchronous or metachronous polyps: classical adenomas (12 patients), sessile serrated adenoma/SSA (1 patient), hyperplastic polyps/HPs (7 patients), both HPs and adenomas (6 patients) and colorectal cancer (2 patients). Size of the lesions varied from 1 to 6 mm (mean: 3 mm). Histologically, all lesions were composed of serrated epithelial crypts that were separated and distorted by spindle cell stromal proliferations (consistently EMA+, claudin-1+ and GLUT-1+). The epithelial component displayed features of HPs ($n = 17$) and SSA ($n = 4$). Laser-microdissection-guided molecular testing was successful for 13 epithelial and 9 stromal components (9 paired samples). The *BRAF V600E* mutation was detected in 54% of the epithelial but in none of the stromal components. In conclusion, colorectal FPs represent genuine serrated epithelial polyps corresponding either to HP or (less frequently) SSA and should be better classified as such with a note on the presence of the stromal component. A more concise terminology reflecting their epithelial nature is needed to fulfill the requirements for colorectal cancer risk assessment and hence adopt appropriate follow-up strategies.

1. Introduction

The term "*fibroblastic polyp*" (FP) was coined by Eslami-Varzaneh in 2004 for minute solitary polypoid colorectal mucosal lesions composed of bland slender mesenchymal cells within the lamina propria mucosae without involvement of the adjacent submucosa [1]. Although classical adenomas and hyperplastic polyps (HPs) located at different sites of the large bowel were present in the majority of cases, no association with an identifiable polyposis syndrome was observed [1]. Only three of the 14 cases in the original report were closely associated with HPs [1]. Based on essentially negative immunohistochemical findings and on ultrastructural features in two lesions, a fibroblastic line of differentiation was favored by the authors justifying the term "fibroblastic polyps" [1]. In 2005, Hornick and Fletcher described a series of 10 intestinal perineuriomas; 8 of them were identical to FPs reported previously and 5 of these 8 lesions were associated with hyperplastic mucosal crypts [2]. Soon thereafter, consistent association of FPs with crypt serration and their identity with mucosal perineuriomas have been postulated by Groisman et al. and those cases initially considered EMA-negative were reportedly positive after reassessment [3-5]. Consistent with a neoplastic nature of the serrated epithelial component, Agaimy et al. demonstrated regular association of FPs with serrated crypts in a series of 29 lesions and detected *BRAF V600E* (63%) and *KRAS* (4%) mutations in majority of cases [6]. Another study by Pai et al. demonstrated the *BRAFV600E* mutation in 92% of sessile serrated adenomas (SSAs) with perineurial-like proliferations and in all 18

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serrated colorectal perineuriomas but in none of two non-serrated perineuriomas [7]. In the current study, we describe clinicopathological and molecular features of 21 serrated FPs. We examined the epithelial and stromal components separately for the presence of *BRAF* mutations.

2. Material and methods

Cases have been retrieved from our routine and consultation files. All have been initially diagnosed on Hematoxylin and Eosin (H&E) stained slides as FPs according to the presence of typical histological features in the mesenchymal component irrespective of the presence or absence of serrated mucosal crypts within the lesion. All lesions have been re-reviewed to confirm diagnosis and to classify the epithelial component as either HP or SSA. SSA is defined by the presence of two or more crypts showing the defined morphological features according to the German consensus [8,9]. Further endoscopic findings were obtained from the clinical records and previous pathology reports. Immunohistochemistry was performed on 3 µm sections using a polymer Kit purchased from Zytomed systems Ltd., Berlin, Germany according to the manufacturer's instructions and the following antibodies: epithelial membrane antigen/EMA (clone E29, 1:200, DakoCytomation; microwave pretreatment), Glucose transporter 1 (GLUT-1, polyclonal, 1:200, ThermoScientific, Fremont, CA 94539 USA) and claudin-1 (polyclonal, 1:100, Zytomed). Immunohistochemical assessment was done as reported previously for FPs. Cytoplasmic and perimembranous staining of any extent was considered positive [6].

2.1. Microdissection and DNA isolation

For molecular analysis, 5 µm sections were cut from formalin-fixed paraffin embedded tissue specimens and stained with Hematoxylin and Eosin (H&E) for visualization of the different components for microdissection. Based on the quantity and distribution of the stromal and epithelial component, either component was microdissected under view of laser-capture microscope followed by microdissection of the remaining component (Fig.1). Any contamination with non-serrated epithelium or other mesenchymal components of the lamina propria mucosae was strictly avoided. DNA isolation was performed using the QIAamp DNA FFPE Tissue Kit (QIAGEN, Hilden, Germany) according to manufacturer's instruction.

2.2. BRAF mutation analysis using dideoxysequencing

Mutations carried by codon 600 of the *BRAF* gene were analyzed using two successive PCR amplifications. Nested PCR is mandatory in view of the very small amount of DNA obtained after microlaser capture. Following PCR primers were used: First PCR: BRAF_15F: 5′-TAC CTAAACTCTTCATAATGCTTGCTCTG-3′;

Braf_15R: 5′-GCCTCAATTCTTACCATCCACAAAA-3′; nested PCR: Braf_15Fn: 5′-CTTCATGAAGACCTCACAGTAAAAATAGG-3′; Braf_15Rn 5′-TAGCCTCAATTCTTACCATCCACAAA-3′. Experimental condition of the first PCR and nested PCR were identical and as follow: 39 cycles, denaturation at 95 °C for 25 s, annealing at 56 °C for 10s and elongation at 72 °C for 1 min. A final elongation time for 7 min was done consecutively to the amplification. The excess of primers and nucleotides was enzymatically eliminated by adding a volume of 20% of exonuclease and shrimp alkaline phosphatase, ExoSap (Affymetrix #78201). Sequencing PCR was performed using single nested primers as follows: 50 cycles, denaturation at 96 °C for 10 s, annealing at 55 °C for 10 s and elongation at 60 °C for 1.40 min. Capillary electrophoresis was done on an ABI 3130 Genetic Analyzer and sequences analyzed using the software Sequencing Analysis 5.2 (Applied Biosystems).

3. Results

The study group comprised a total of 21 lesions from 19 patients (Table 1). There were 10 females and 9 males aged 45 to 80 yrs. (mean, 62 yrs). Mean age was 60.8 yrs. and 63.7 yrs. for women and men, respectively. Lesions were detected in the sigmoid colon/rectosigmoid junction (n = 16), rectum (n = 2), ascending colon (n = 2) and descending colon (n = 1). All but two lesions originated distal to the left colonic flexure and of them, 18 lesions were localized to the rectosigmoid colon (94.7%). In 13 patients, the FPs were associated with other type of colorectal epithelial polyps removed either simultaneously or metachronously. Classical colorectal adenomas were seen in 12 patients and sessile serrated adenoma (SSA) in 1 patient (multiple SSA in different parts of the colon). Also, HPs were a common bystander, being detected in 7 patients (of them 2 patients had 3 or more HPs). Two patients had invasive colon cancer (both proximal to the splenic flexure) in addition to tubular adenomas, HPs and other epithelial polyps (see Table 1).

3.1. Histopathological and immunohistochemical features of fibroblastic polyps

Size of the lesion varied from 1 mm to 6 mm (mean: 3 mm). Histologically, all lesions presented as small slightly elevated polyps composed of mucosal crypts that were widely separated and distorted by a bland looking spindle cell mesenchymal proliferation (Fig. 2A-E). The epithelial component showed architectural and cellular features of HPs in 17 lesions with prominently serrated superficial crypts and hyperchromatic rounded non-serrated basal crypts featuring regenerative changes. Four FPs displayed two or more crypts with basal crypt serration/dilatation, prominent goblet cells in the basal crypts, horizontal and branched crypts or other features of SSA. Notably, the FPs of both patients with invasive cancer had SSA-conform epithelial component. There was no evidence of atypia, mitotic figures or infiltrative growth. None of the lesion extended to the submucosa and none showed

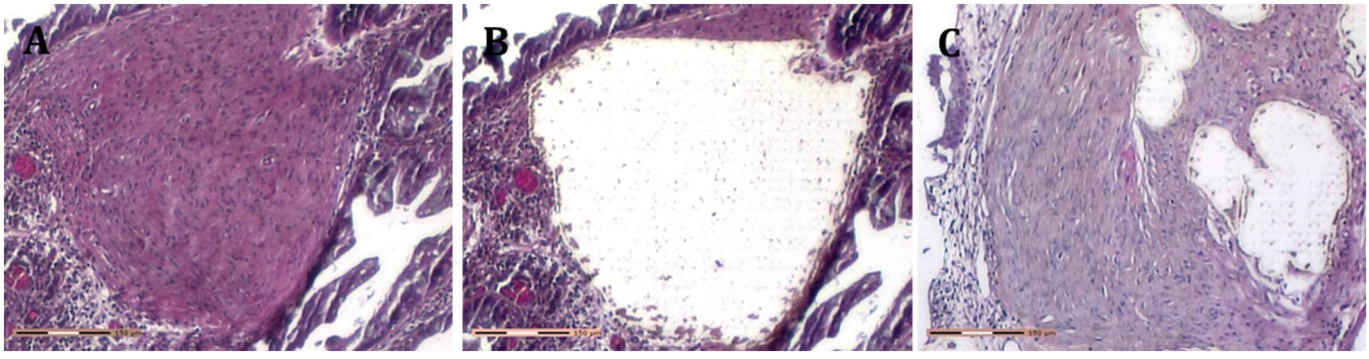

Fig. 1. Laser capture microdissection was performed using thick HE-stained sections to visualize the two different components (A) followed by marking and microdissecting either the stromal (B) or the epithelial (C) component followed by dissecting the remaining tissue.