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MSI-H 'medullary type' adenocarcinoma complicating ileal Crohn's disease; further molecular insight into Crohn's related carcinogenesis.

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MSI-H 'medullary type' adenocarcinoma complicating ileal Crohn's disease; further molecular insight into Crohn's related carcinogenesis.

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Sir: We present the case of a 63-year-old man who presented in 1998 with symptoms of small bowel obstruction. Mid small bowel strictures were noted on CT scan and with contrast radiology, and a clinico-radiological diagnosis of Crohn's disease (CD) was made. Symptoms improved without the need for medical or surgical intervention. There was no family history of colorectal cancer. He represented in 2007 with abdominal pain, weight loss, and signs of small bowel obstruction. At laparotomy a 350mm length of ileum was resected showing extensive diffuse mural thickening, fat wrapping, and two distinct strictures, the longer 90mm, all thought macroscopically to represent CD (Fig. 1a). At surgical cut up sectioning of the longer stricture revealed diffuse replacement of the bowel wall by firm pale yellowish tissue with well defined pushing borders (Fig. 1b). Representative sections taken from along the small bowel revealed foci with typical histological features of long standing CD comprising patchy mucosal active inflammation, fissuring ulceration, foci of ulcer associated cell lineage (UACL), and transmural fibrosis and inflammation with a granulomatous component. Sections from the longer stricture revealed a well-circumscribed, poorly differentiated carcinoma (Fig. 1c) comprising cells with abundant eosinophilic cytoplasm, round vesicular nuclei and prominent nucleoli, reminiscent of the so-called medullary variant of adenocarcinoma (AC)(Fig. 1d). There was a major inflammatory component including numerous lymphocytes. Convincing background precancerous dysplasia was seen which was adenoma-like without serration

(confirmed on review by Prof J Jass). Immunostaining was positive for pankeratin, CEA, and cytokeratin 7 (CK 7) (Fig. 2), whereas CK20, EMA, AFP, CDX2 (Fig. 2) and neuroendocrine markers were negative (usual phenotype for CRC is CK20 pos. CK 7 neg). Immunostaining of tumour for mismatch repair (MMR) gene proteins MSH-2 and MLH-1 revealed loss of nuclear staining with anti MLH-1 (Fig. 2). Screening of tumour DNA revealed replication errors in all of the 7 microsatallite markers tested (BAT25, BAT26, BAT40, D2S123, D5S346, D8S255 and D17S250), stratified as microsatellite instability-high (MSI-H). Sequencing of exon 15 of BRAF using DNA amplified from tumour tissue showed no evidence of the p.Val600Glu (V600E) activating mutation.

The increased risk of colorectal AC in CD is well recognised [1,2,3] and equal to ulcerative colitis (UC). Although less common, small bowel AC in the same setting is well reported since first described by Ginzburg et al in 1956 [4-9]. As in our case clinical presentation is typically 'occult' in line with a recent study reporting a preoperative diagnosis in only 1 in 20 cases in patients with long standing CD as compared with 22 of 40 patients with sporadic disease [10]. A number of population and hospital based studies together with case reports show an increased risk of small bowel AC in CD with results varying between 3-fold and 91-fold increased risk, but based only on a few cases in each series [3]. Only one study describes relative risk in patients with ileal disease at first diagnosis [10] with cumulative risk at 10 years reported as 0.2%, but as 2.2% at 25 years.

The increased risk of malignancy in inflammatory bowel disease (IBD) is ascribed to factors resulting from chronic inflammation [11]. Most cases of small bowel AC in this setting have dysplasia in adjacent mucosa supporting a dysplasia- carcinoma pathogenesis [9]. Much of the current understanding of the molecular alterations involved in the development of neoplasia in IBD come from studies of patients with UC, but are probably valid in part for CD. In contrast to sporadic colonic AC aneuploidy and p53 mutation are early events in UC related dysplasia to carcinoma progression, whereas APC mutation, frequently seen early in sporadic cancer progression, is less frequent and late in the UC setting [12]. As many as 15%-40% of UC patients demonstrate MSI in cancer tissues, with MSI-H neither under or overrepresented. Oxidative stress can functionally impair the protein components of the mismatch repair gene (MMR) system without necessarily causing gene mutations, which may contribute to the MSI-low phenotype seen in UC [13]. There is conflicting data on the finding of germline *hMSH2* mutations in UC [14,15], and promoter methylation, an important mechanism for silencing of the hMLH1 gene in sporadic CRC, seems to contribute less to UC tumorigenesis [16]. In our patient gene mutation analysis had not been carried out at the time of writing.

The morphology of the tumour presented here closely resembles the recently described distinct class of poorly differentiated large bowel AC often termed medullary-type (MTA) [17,18,19]. Furthermore these tumours frequently show microsatellite instability (MSI) by molecular genetic analysis [20,21] suggesting the existence of a distinct type of AC, sporadic or associated with hereditary non-polyposis colorectal cancer (HNPCC), with clearly defined clinicopathological and biological characteristics [22]. A recent review by Jass

[23] proposes a classification of colorectal cancer (CRC) based on the clinical, morphological and molecular features. Poorly differentiated AC are overrepresented in type 1 CRC (CpG island methylator phenotype-high ([CIMP-high]/MSI-H/BRAF mutation) and type 5 or Lynch syndrome CRC (MSI-H). Medullary AC occurs in both type 1 and Lynch syndrome CRC, but is rare in both. Type 1 CRC do show a degree of morphological heterogeneity however. The homebox gene *CDX2* is mutated in MSI-H CRC but gene mutation was observed in only 3.2% of Lynch syndrome CRC [23,24]. Loss of CDX2 expression is associated with medullary CRC [25,26], and loss of CDX2 immunostaining in tumour tissue was a feature in our case (Fig1d). Our tumour resembles sporadic CRC with CIMP-high, comprising cells with round vesicular nuclei, prominent nucleoli and abundant pink cytoplasm. On the basis of this interesting case (and as characterised in the recent review by Jass [23]) one can infer that the typical morphology of type 1 and type 2 CRC is determined by three independent factors;

1) BRAF accounts for serration
2) CIMP-high accounts for poor differentiation and typical nuclear changes
3) MSI-H (type 1 CRC) accounts for lymphocytes (and possibly poor differentiation.

CIMP and BRAF mutation are tightly correlated [23]. The lack of hyperplastic polyps in the small bowel is due to lack of either BRAF or KRAS mutation in this site. However CIMP may develop in small bowel independently of BRAF mutation. CIMP status is unknown in our case.

In summary we have presented the first comprehensive description of a rare distinct medullary variant of AC arising in the small bowel in the setting of long standing Crohn's disease, which shares the morphological and some of the molecular characteristics of a small subgroup of sporadic and hereditary CRC. This case in part fits with the recently proposed classification of CRC based on clinical, morphological and molecular features, and gives further insight into the pathogenesis of carcinoma in the setting of IBD, and in particular, CD.

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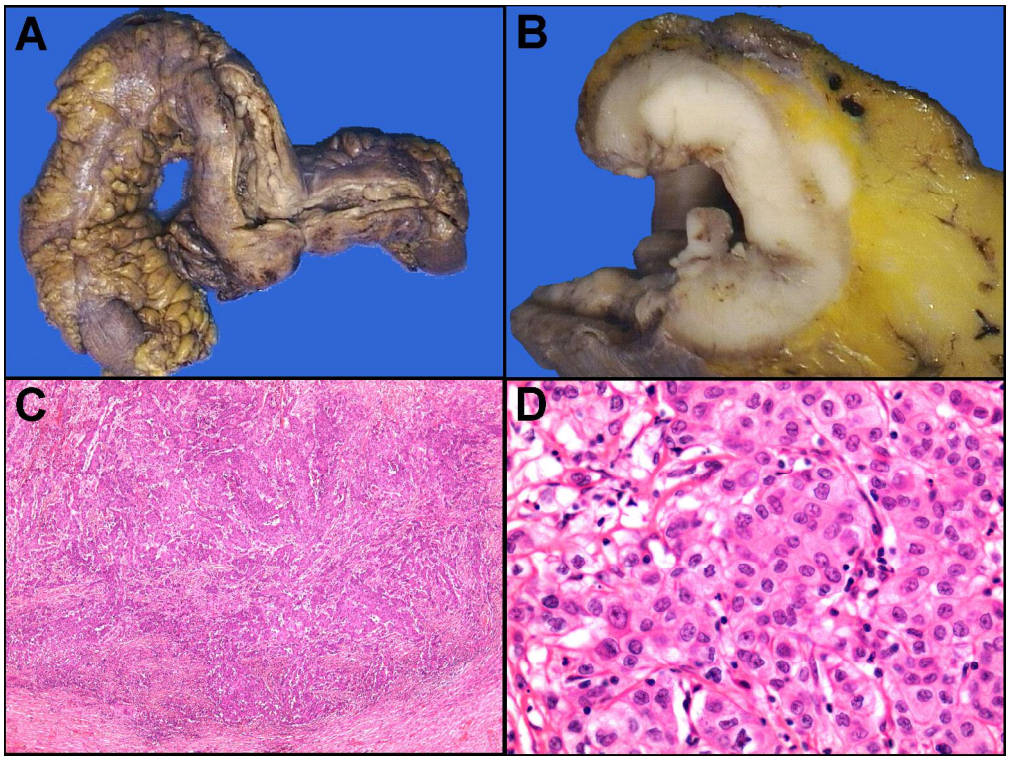
Legends

Fig 1. A 350mm length of ileum showing fat wrapping (left field) and strictures (A) Transverse section of small bowel stricture showing intramural circumscribed yellowish tumour (B) Poorly differentiated carcinoma H & E x200 (C) comprising cells with abundant eosinophilic cytoplasm, vesicular nuclei with prominent nucleoli (D)

Fig. 2 Tumour immunostaining showing strong positive staining with CK7, loss of tumour CDX2 expression compared with normal small bowel (right field), MSH-2 gene protein expression in tumour cell nuclei but loss of nuclear MLH-1 gene protein expression.



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273x204mm (300 x 300 DPI)

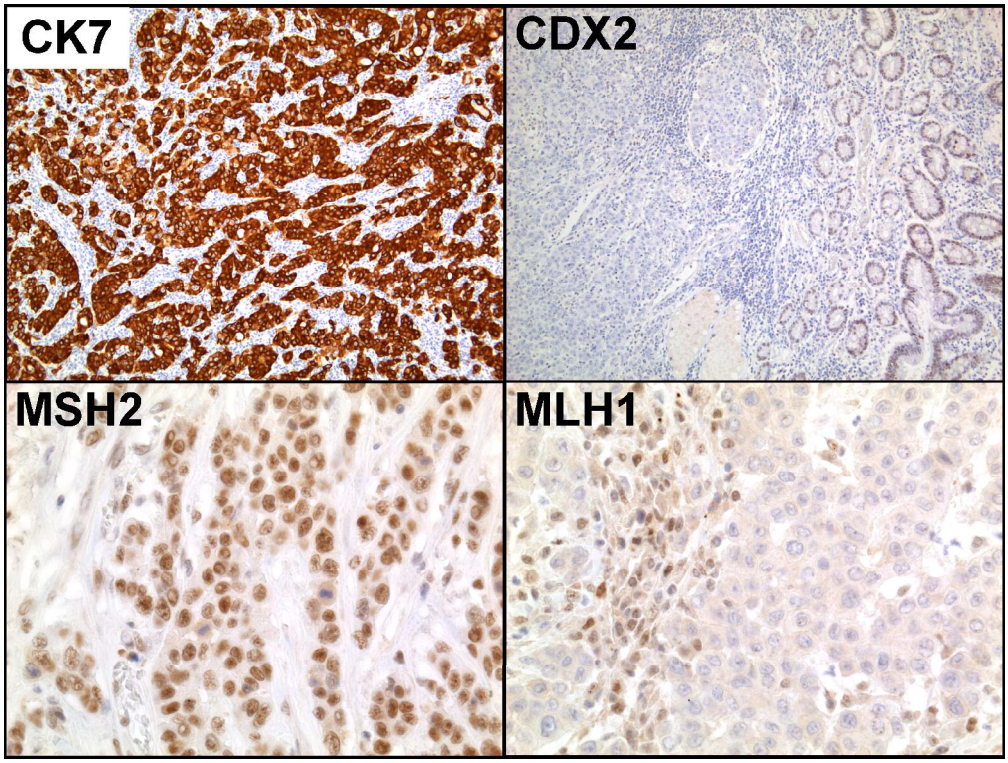

Fig. 2 Tumour immunostaining showing strong positive staining with CK7, loss of tumour CDX2 expression compared with normal small bowel (right field), MSH-2 gene protein expression in tumour cell nuclei but loss of nuclear MLH-1 gene protein expression.
273x206mm (300 x 300 DPI)