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ANATOMICAL PATHOLOGY

Adenocarcinoma in Crohn's disease: the pathologist's experience in a tertiary referral centre of inflammatory bowel disease

Deborah Malvi[1], Francesco Vasuri[1], Benedetta Mattioli[2], Elisa Gruppioni[1], Michelangelo Fiorentino[1], Paolo Gionchetti[3], Walter F. Grigioni[1], Gilberto Poggioli[2] and Antonia D'Errico-Grigioni[1]

[1]'F. Addarii' Institute of Oncology and Transplant Pathology, [2]Department of Surgery, and [3]Inflammatory Bowel Disease Unit, S. Orsola-Malpighi Hospital, Bologna University, Bologna, Italy

Summary

The aim of the present study is to describe the histological and mutational characteristics of a series of both large and small bowel adenocarcinomas in patients with Crohn's disease from a tertiary referral centre of inflammatory bowel disease. Bowel adenocarcinoma was diagnosed in 11 (1.7%) of 660 consecutive patients submitted to surgery for histologically proven Crohn's disease in 5 years. The following data were collected: tumour site, stage and grade, intracellular/extracellular mucin, lymphovascular invasion, immunohistochemistry for keratin 7, keratin 20 and CDX-2, mutation analyses of KRAS, B-RAF, PI3K and microsatellite instability. A strong predominance of male gender was observed (10/11). Four (36.4%) adenocarcinomas arose in the small bowel, five (45.4%) in the anus/rectum, and two (18.2%) in anastomosis. Furthermore, all cases of anorectal adenocarcinoma showed >50% of extracellular mucin, with associated KRAS mutations in three of five. No influence in cancer incidence by infliximab therapy was observed. Our series, one of the largest on the topic with immunomorphological and molecular deepening, showed that bowel adenocarcinomas in Crohn's disease have an aggressive behaviour and a strong predominance of extracellular mucin. In surgical specimens from Crohn's disease patients, mucinous-looking anal fistulas and ileal areas of adhesion/retraction should always be extensively sampled.

Key words: Adenocarcinoma, Crohn's disease, histopathology, molecular biology, mucinous adenocarcinoma.

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INTRODUCTION

Patients with inflammatory bowel disease (IBD) are at an increased risk of developing intestinal cancers. Some papers have reported that colorectal cancer in patients with Crohn's disease (CD) has an increased incidence of 2.9% 10 years after the onset of the disease, and 8.3% after 30 years, with a 10- to 40-fold increased risk compared to the general population.[1,2] Also, small bowel adenocarcinoma arising in the setting of CD is a well-known complication, with an increased risk compared to the general population.[3,4] Nevertheless, the incidence of small bowel adenocarcinoma in patients with CD is still low (1.6%),[5] and the relatively high risks seem to be mainly related to the very low incidence of small bowel adenocarcinoma in the general population.[3,4] The most problematic issue is that neoplasia and inflammation can induce the same occlusive symptoms. Moreover, the imaging signs of cancer and CD are often identical. This explains the high stage of these tumours at the time of surgery:[6–8] up to 35% of adenocarcinomas are diagnosed at stage IV.[3]

It is now widely accepted that the condition IBD leads to an increased intestinal cancer risk through a different multi-step process that includes inflammation, dysplasia and cancer, since these cancers appear to arise from either flat foci of dysplastic tissue or dysplasia-associated lesions or masses (DALM). This is particularly true for IBD/ulcerative colitis, while the molecular cancer pathways in CD are not completely understood yet, since the 'classic' adenoma-cancer sequence seems not to be the main pathogenetic mechanism involved.[2,9]

Intestinal adenocarcinoma represents a rare complication of CD, found during surgical resection for active CD disease. In the last decade, only single cases of anorectal adenocarcinomas, sometimes arising in the background of complex fistulas, have been reported in the literature,[10–17] while the few single or multicentric studies on small bowel adenocarcinoma in CD have dealt with the problem from a clinical and therapeutic point of view.[3–6,18,19] The aim of this study was to describe the morphological aspects of adenocarcinoma arising in CD surgically treated in a tertiary referral centre of IBD. We observed all cases of both large and small bowel adenocarcinomas in CD patients in a 5-year time period. Our purpose was to delve into the histological and mutational characteristics of these cancers, together with their biological behaviour.

MATERIALS AND METHODS

Case selection and histopathological analysis

We reviewed the clinical records of 777 patients (365 females, 47.0%; 412 male, 53.0%) submitted to surgery for CD over a 5 year period (2007–2011) in the Surgical Unit of S. Orsola-Malpighi Hospital, Bologna, Italy. In 660 cases the diagnosis of CD was histologically confirmed by a prior biopsy and/or on resected specimens, while in the other cases patients were submitted to conservative bowel surgery (strictureplasty) or perianal drainage, and therefore no histopathological examination was performed.

After the diagnosis of CD, 258 of 660 (39.1%) patients received biological therapy with infliximab. In particular, 221 received systemic therapy alone for ileo-colic CD, while 62 with complex perianal CD received a local injection of infliximab ($n = 49$) or both local and systemic treatment ($n = 12$), according to the protocols applied in our Centre.[20]

Eighteen patients had a histopathological diagnosis of abdominal neoplasia, among which 11 were diagnosed as intestinal adenocarcinomas (which

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represents 1.7% of the patients with histologically proven CD); these 11 patients were included for the purposes of this study. The remaining seven abdominal neoplasms—which were not included in the study—proved to be one malignant peripheral nerve sheath tumour (MPNST), one diffuse large B-cell lymphoma (DLBCL), two neuroendocrine tumours (NET), one endometrial adenocarcinoma, one ovarian carcinoma and one bladder carcinoma, all of them infiltrating the large bowel. Eight patients had high grade or low grade dysplastic foci which were found on colon surgical resection in all CD patients; two were associated with adenocarcinoma (18.2% of CD patients with bowel adenocarcinoma) and six in CD patients without bowel adenocarcinoma (0.9% of CD patients without bowel adenocarcinoma).

The clinical data on CD activity and therapy, the presentation of neoplastic disease, and the follow-up data (i.e., neoplastic recurrence and survival) were recorded for all 11 patients with intestinal adenocarcinoma. For each case of intestinal primary malignancy, the following histological parameters were evaluated on surgical specimen: tumour site, TNM tumour classification according to the American Joint Committee on Cancer (AJCC) 2010,[21] histological grade according to the World Health Organization (WHO), presence and amount of intracellular and extracellular mucin according to WHO (absent, <50%, or >50%) and the presence of lymphovascular neoplastic invasion.[22]

Immunohistochemistry

Immunohistochemistry (IHC) for keratin 7 (K7), keratin 20 (K20) and CDX-2 was automatically performed in the BenchMark XT automated stainer (Ventana, USA). Sections 3 μm thick were cut from formalin fixed, paraffin embedded (FFPE) specimens, mounted on positively charged slides (SuperFrost Plus; Menzel, Germany) and baked overnight at 37°C. K7, K20 and CDX-2 IHC stainings were performed with prediluted monoclonal antibodies (Cytokeratin 7, clone SP52, Ventana; Cytokeratin 20, clone SP33, Roche, Switzerland; CDX-2, clone EPR2764, Cell Marque, USA) in the BenchMark XT automated stainer (Ventana) using the Ultraview DAB Detection kit (Ventana). Negative controls were performed by omitting the primary antibodies. Positivity was semi-quantitatively assessed as strong/diffuse (++), focal and/or weakly positive (+), and negative (–).

Mutational analysis and microsatellite instability

Genomic DNA was extracted from FFPE tissue using the QIAamp DNA Micro Kit (Qiagen, Germany) according to the manufacturer's instructions. Concentration of the extracted DNA was assessed by real time polymerase chain reaction (PCR) using the Quantifiler Kit (Life Technologies, USA). Mutational analyses in oncogenes KRAS (exon 2),[23] BRAF (exon 15)[24] and PI3K (exons 9, 20)[25] were performed using the Direct Sanger Sequencing method, which is able to detect up to 25% of mutated DNA on a background of genomic wild-type DNA. Sequencing analysis was performed using the Automated Sequencer (3730XL Genetic Analyzer; Applied Biosystems, USA), and sequencing results were interpreted with Chromas Software version 1.45 (Technelysium, Australia).

Microsatellite instability (MSI) analysis was performed through PCR reaction, using the CC-MSI kit (AB Analitica, Italy) according to the manufacturer's instructions. This kit is designed to co-amplify 10 markers (BAT25, BAT26, D2S123, RII and D18S58) in two BD5S346, D17S250, NR21, NR24, BAT40, TGF separate reactions. The fluorescent amplified PCR products (from normal and tumour tissue) were analysed by capillary gel electrophoresis on an ABI 3730XL DNA Analyzer (Life Technologies), using the GeneMapper software, version 4.0 (Life Technologies). Tumours were classified as MSI-high (MSI-H; 4/10 markers showing MSI), MSI-low (MSI-L; 1–3/10 markers showing MSI), or MS stable (MSS; no markers showing instability).

Ethical considerations

This study conformed to the ethical guidelines of the 1975 Helsinki Declaration as amended in 1983, 1989, 1996, and 2000, using archival tissue from routine histological examination. Informed consent was obtained from the patients at the time of surgery.

RESULTS

Patients and clinical data

The 11 patients with intestinal adenocarcinoma were 10 males and one female, and mean age at the time of cancer diagnosis was 50.6 ± 12.5 years (range 31–76 years). All 11 patients were previously treated with total colectomy for CD activity.

The mean time from CD diagnosis to cancer diagnosis was 23.4 ± 11.6 years (range 1–47 years). Four of 11 (36.4%) received biological therapy (2 local injection and 2 systemic therapy), after a mean time of 20.3 ± 28.1 months from CD diagnosis (range 1.9–62.1 months). These patients represented 1.6% of the 258 treated patients. The patients who did not receive infliximab showed the same incidence of intestinal adenocarcinoma (7/402 cases, 1.7%). The clinical onset of the neoplastic disease was not specific for cancer-related symptoms. Patients with small bowel or ileo-colic adenocarcinomas underwent surgery for occlusive symptoms, since no neoplastic lesions were suspected at the moment of surgery. At gross examination, an ulcerative stenotic lesion with firm whitish neoplastic tissue was unexpectedly found infiltrating the visceral wall, often with adhesions.

The patients with anorectal adenocarcinomas generally underwent surgery for complex perianal CD. Suspicion of malignancy was raised by the surgeons in two of five cases at the time of resection, and a frozen section intraoperative analysis was required in one case (with a diagnosis of mucinous adenocarcinoma). In all cases the gross examination revealed a fistula filled with mucoid material.

After a mean follow-up of 1.7 ± 1.1 years after cancer diagnosis (range 0.4–4.2 years), five (45.5%) patients died of cancer.

Histology and immunohistochemistry

Table 1 summarises the clinical, morphological, IHC and mutational features of the 11 adenocarcinoma cases.

Seven adenocarcinomas were graded as grade 3 according to WHO, three as grade 2 and one as grade 4 (undifferentiated). Four were classified as pT4a, three as pT4b, and four as pT3; one case was pN2a, four were pN1, and six pN0. Distant metastasis (pM1b) were present in two cases at the time of the diagnosis. Eight cases had vascular/lymphatic neoplastic invasion. In two cases the surgical resection was not radical. Extracellular mucin >50% of neoplastic area was described in seven cases, <50% in 1 case, and absent in three cases. Intracellular mucin (with a 'signet ring' appearance) was present in five cases. Foci of low grade adenomatous dysplasia were observed in two (18.2%) cases in the remaining bowel examined, while in three (27.3%) cases foci of pyloric metaplasia were found in the non-neoplastic mucosa adjacent to the adenocarcinoma.

IHC positivity for CDX-2 was recorded in all cases. K20 was strongly and diffusely positive in seven cases (63.6%), focally positive in three cases (27.3%) and negative in one case (9.1%, the undifferentiated carcinoma). K7 was strongly and diffusely positive in one case (9.1%), focally positive in three cases (27.3%) and negative in seven cases (63.6%). Finally, seven of 11 adenocarcinomas, including two arising in the small bowel, showed a K20+/K7–/CDX2+ profile, four adenocarcinomas showed a K20+/K7+/CDX2+ profile (Fig. 1), while one case (undifferentiated adenocarcinoma) was positive for CDX2 only (Table 1).

Mutational analysis and microsatellite instability

All cases in our series were BRAF and PI3K wild-type. Seven (63.6%) cases were KRAS wild-type. The four (36.4%) mutated cases encompassed the following exon 2 mutation