CASE REPORT

Synchronous colorectal neoplasms in ulcerative pancolitis of 6 years duration: pathological and molecular heterogeneity

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SUMMARY

Colorectal cancer (CRC) complicating ulcerative colitis (UC) accounts for about 1% of all cases of CRC. Such tumours develop from pre-existing foci of dysplasia in patients with extensive and long-standing UC. We report a case of UC-associated synchronous CRC and foci of high-grade dysplasia with an additional malignant focus in the appendix in a female patient after only 6 years of pancolitis who did not have other risk factors for the development of complications. The multiplicity and the timings of the early changes noted suggest that long-standing inflammation in UC randomly damages multiple genes in epithelial cells known to contribute to colorectal carcinogenesis. Current findings also support a molecular and pathological heterogeneity during multiclonal origin of synchronous tumours whereby differences occur at various sites that were absent during the initial stages of the disease.

BACKGROUND

Ulcerative colitis (UC) patients have an at least twofold increase in the lifetime risk of developing colorectal cancer (CRC) compared with the normal population, and this complication is observed in about 5.5–13.5% of cases.¹ The risk factors for CRC in UC include disease extent and duration, primary sclerosing cholangitis, tobacco smoking, family history of CRC, as well as continuing inflammatory activity. Different professional societies have guidelines for screening for CRC in UC patients that recommend surveillance beginning at 8–10 years after the onset of symptoms in patients with disease upstream to the rectum.² Synchronous and multifocal cancers are more common in UC than sporadic CRC with a frequency of 10–30%, but the molecular background, which could potentially help in early detection, remains poorly explained.^{3–4} We report a case of UC-associated synchronous colorectal carcinoma with a malignant focus in the appendix in a patient with UC of 6 years, which showed pathological and molecular heterogeneity in different areas of the cancer.

CASE PRESENTATION

A 16-year-old girl presented to us in 2008 with recurrent, bloody diarrhoea of 2 years' duration. The patient was diagnosed to have severe UC based on typical clinical presentation, sigmoidoscopic findings and histological features after ruling out infectious causes. She was a non-smoker and there

was no family history of CRC. She continued to have severely active disease on corticosteroids initially, refused proctocolectomy and was managed with intravenous and later oral cyclosporine bridging over to azathioprine after 3 months. The patient reported keeping fairly well during follow-up visits but a persistent iron deficiency anaemia detected 18 months later prompted a colonoscopic evaluation. This revealed extensive colitis with severe endoscopic disease activity (Baron Grade IV); there was no dysplasia on the colonoscopic biopsies. Azathioprine was continued, the dose of 5-amino-salicylic acid increased and iron supplements were added. Anaemia and endoscopic disease activity persisted over the next 6 months but the patient opted for continued medical therapy rather than proctocolectomy because of negligible bowel symptoms.

In 2011, 6 years after the onset of colitis, she presented with recent, right-sided abdominal pain of 10 days. Physical examination was remarkable only for anaemia and a poorly defined, non-tender mass in the right lower quadrant of the abdomen. Laboratory parameters were non-contributory. A CT scan revealed a circumferential, asymmetrical wall thickening with contrast enhancement involving the ascending colon and hepatic flexure, and a few areas of focal thickening of the wall of the large bowel downstream. A fluid collection suggestive of an abscess was also noted in the right iliac fossa in addition to changes of long-standing extensive ulcerative colitis. Colonoscopic biopsies from the stricture at the hepatic flexure revealed moderately differentiated adenocarcinoma. There were foci of high grade dysplasia, and of adenocarcinoma in the biopsies from the areas of focal mucosal thickening or nodularity found downstream to the stricture. At laparotomy done because of worsening symptoms, there was evidence for a contained perforation of the appendix, a mass lesion involving the ascending colon and multiple malignant deposits on the peritoneal surface of the colon; total colectomy with ileostomy was performed. Histology highlighted well-differentiated mucinous adenocarcinoma with anaplastic regions in at least three separate areas of the large bowel. An independent focus of adenocarcinoma in the appendix was also seen. In addition, villous glandular adenoma with high-grade dysplasia was found in the flat and raised areas on a background of long-standing UC.

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Table 1 Results of Immunohistochemistry analysis carried out on the four different areas from the colectomy specimen

Gene	CRC mucinous area	CRC anaplastic area	Villous area (HGD)	Villous polyploid area (HGD)
P53	+++	+	+++	+++
C-MYC	++	—	+	+
CCND1	+	+++	+	++
EGFR	—	+++	+	+
ERBB2	—	—	—	—
AMACR	—	—	—	—
Ki67 (%)	30	20	20	20

CRC, colorectal cancer; HGD, high-grade dysplasia; —, negative immunostaining; +, weak immunostaining; ++, moderate immunostaining; +++, strong immunostaining.

INVESTIGATIONS

Molecular and immunohistochemical analysis

Immunohistochemistry (IHC) for p53, ki67, ERBB2, EGFR, CCND1, C-MYC and AMACR were performed on four different regions from the colectomy specimen—mucinous adenocarcinoma, anaplastic carcinoma and villous flat and polypoid areas, the latter two showing high-grade dysplasia. DNA extracted from endoscopic biopsies collected from the patient at three different earlier time points as a part of another study—during initial sigmoidoscopy performed for diagnosing UC, full-length colonoscopy performed about 1.5 years later for assessing disease activity and extent and the last colonoscopy performed to assess the stricture—were available for analysis of molecular markers of neoplasia. DNA extracted from the peripheral blood drawn at the time of diagnosis of cancer by colonoscopy was used for the analysis for molecular changes. *p53* (exons 4–8), *KRAS* (codons 12 and 13) and *BRAF* (codon 600)

mutations were analysed in these samples using PCR-sequencing method. Microsatellite instability (MSI) analysis was carried out using NCI-Bethesda panel of markers by capillary electrophoresis method.⁵ Amplification of the oncogenes *C-MYC*, *MYCN*, *CCND1*, *CCND2* and *EGFR* was assessed using TaqMan assay-based real-time PCR method.

On IHC, the mucinous adenocarcinoma showed moderate-to-strong p53 and C-MYC staining but weak staining for CCND1 and no staining for EGFR. The anaplastic region showed moderate to strong staining for CCND1 and EGFR genes and very weak staining for the p53. In contrast, the villous flat and polypoid areas, which harboured HGD, showed strong p53 staining with weak staining for CCND1, EGFR and C-MYC. Staining for the proliferative marker Ki67 was the same across all the four areas. All areas were negative for ERBB2 and AMACR markers (table 1 and figure 1).

In the molecular analysis carried out on the sigmoidoscopic and colonoscopic biopsies obtained at 2 and 4 years after the onset of disease, none of the molecular alterations analysed showed any changes. The biopsy samples obtained at the last colonoscopy showing anaplastic areas harboured a heterozygous deletion of the C at codon 178 on the *p53* gene resulting in a stop codon (figure 1). The *MYC* gene was amplified in malignant areas as well as in the areas with neoplasia. For the same set of biopsies the other markers analysed in the present study did not show any alterations. The blood sample taken during colonoscopy when CRC was diagnosed did not show any molecular alterations (table 2).

OUTCOME AND FOLLOW-UP

The patient was discharged to a local hospital for palliative care.

DISCUSSION

The reported case highlights a number of unusual features. The patient developed an aggressive multifocal colon cancer in the

Figure 1 (A) Macroscopic view of the resected colon specimen; (B1) p53 heterozygous C deletion in stricture; (B2, B3 and B4) positive immunostaining for p53, EGFR and CCND1 genes, respectively; (C) Timeline of clinical, pathological and molecular events during follow-up.

Table 2 Results of molecular analysis carried out on the samples taken during follow-up colonoscopies

Sampling year (duration of disease)	2008 (2 years)	2010 (4 years)	2012 (6 years)			
Biopsy areas	Random	Random	Random	Stricture	Villous	Blood
Disease activity	Active	Active	Remission			
<i>p53</i> gene						
Exon 4	WT	WT	WT	WT	WT	WT
Exons 5–6	WT	WT	WT	Het del C at codon 178	WT	WT
Exons 7–8	WT	WT	WT	WT	WT	WT
<i>KRAS</i> codon12 &13	WT	WT	WT	WT	WT	WT
<i>BRAF</i> codon 600	WT	WT	WT	WT	WT	WT
<i>MSI</i> analysis	MSS	MSS	MSS	MSS	MSS	MSS
Gene amplification						
<i>C-MYC</i>	Nor	Nor	Amp	Amp	Amp	Nor
<i>N-MYC</i>	Nor	Nor	Nor	Nor	Nor	Nor
<i>CCND1</i>	Nor	Nor	Nor	Nor	Nor	Nor
<i>CCND2</i>	Nor	Nor	Nor	Nor	Nor	Nor
<i>EGFR</i>	Nor	Nor	Nor	Nor	Nor	Nor

Bold signifies the positive alterations in the markers analysed.

WT, wild type; del C, deletion of C allele; MSS, microsatellite stable; Nor, normal (2 copies of the gene) and Amp, amplification (≥ 3 copies). MSI (microsatellite instability) analysis performed using NCI panel of markers; and gene amplification by qRT-PCR analysis.

absence of risk factors commonly reported for colitis-associated cancer such as a long duration of disease, primary sclerosing cholangitis, tobacco smoking and a family history of CRC. Some risk factors were present such as extensive colitis and poorly controlled inflammatory activity but per se these are not deemed to increase the risk of CRC before 8–10 years of UC. UC-associated carcinomas, as compared with sporadic ones, often present as mucinous or signet ring cell adenocarcinoma but only rarely as anaplastic carcinoma.^{6,7} A malignant focus in the appendix, as was seen in our patient, is also not commonly described. The multifocal tumours showed pathological and molecular heterogeneity suggesting differing molecular pathogenesis in different areas. Such molecular heterogeneity and field effects are involved in the development of colitis-associated neoplasia more commonly than in sporadic CRC.⁶ In the IHC study, the area with mucinous carcinoma showed strong expression of p53 along with moderate and weak staining of C-MYC and CCND1, respectively. In contrast, the anaplastic region showed weak p53 expression; whereas CCND1 and EGFR were moderate to strongly expressed and C-MYC was not expressed at all. Such changes have been reported in anaplastic thyroid carcinoma but not well recognised in UC carcinomas. The villous flat as well as polypoid areas showed an expression pattern similar to the areas harbouring mucinous adenocarcinoma. The aggressive nature of the malignancy in our patient might have its explanation in some of the molecular alterations evidenced in the specimen, for example, the C-MYC amplification.⁸

Newly developing strictures are a strong indicator of underlying carcinoma in UC and multiple dysplastic foci are often found in resection specimens following colectomy in such patients, as were seen in our patient.^{9,10} Having less than 8 years of UC, evidence for precancerous lesions were not expected nor specifically sought in this patient on previous evaluations. Nor were these found in the biopsies available from earlier time points and in the molecular analysis done retrospectively on previously collected tissue samples. For example, known markers of neoplasia such as MSI, p53, KRAS or BRAF analysed in the samples obtained before the diagnosis of malignancy were negative. In CRC, mucinous carcinomas are more prone to MSI and BRAF mutations but these changes were not observed in our patient.¹¹

In summary, we report a patient with UC at a low risk for CRC who developed an aggressive multifocal, synchronous form of this complication although no premalignant lesions were noted on previous biopsies from earlier regular follow-up. The lesions showed considerable pathological and molecular heterogeneity which were absent in the initial biopsies. Our findings also support the possibility of the existence of multiple molecular pathways across the different areas of colon and the development of colitis-associated cancers which happened to occur simultaneously in the present case.

Learning points

- ▶ Dysplasia or malignancy in ulcerative colitis (UC) can develop in a patient regardless of age and/or duration of symptoms.
- ▶ Case supports a multiclonal origin of synchronous tumours as the heterogeneity in molecular and pathological phenotypes of the neoplastic lesions differed at various sites.
- ▶ Multiple colonoscopy-directed biopsies combined with molecular and pathological investigation can help in predicting the risk of dysplasia or diagnose malignancy developing in the background of UC.

Contributors KS, CGP and BMSK designed the study, BMSK performed the molecular experiments and analysed the data, CGP performed the clinical and endoscopic evaluation, LR performed all pathological and immunohistochemical characterisations, KS provided the support with molecular analysis. All authors read and approved the final manuscript.

Competing interests None.

Patient consent Obtained.

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