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Field Cancerization in the Intestinal Epithelium of Patients With Crohn's Ileocolitis

Susan Galandiuk[*,‡,§], **Manuel Rodriguez-Justo**[‖], **Rosemary Jeffery**[*], **Anna M. Nicholson**[‡], **Yong Cheng**[*,**], **Dahmane Oukrif**[‖], **George Elia**[¶], **Simon J. Leedham**[#], **Stuart A. C. Mcdonald**[*,‡], **Nicholas A. Wright**[*,‡], and **Trevor A. Graham**[*]

[*]Histopathology Laboratory, Cancer Research UK London Research Institute, London, England [‡]Centre for Digestive Diseases, Blizard Institute of Cell and Molecular Science, Barts and the London School of Medicine and Dentistry, London, England [¶]Centre for Tumour Biology, Institute of Cancer and CR-UK Clinical Centre, Barts and the London School of Medicine and Dentistry, London, England [§]Department of Surgery, University of Louisville, Louisville, Kentucky [‖]Department of Histopathology, University College London Hospital, London, England [#]Molecular and Population Genetics Laboratory, Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford, England [**]Department of Gastrointestinal Surgery, The First Affiliated Hospital of Chongqing Medical University, Chongqing, Peoples Republic of China

Abstract

Background & Aims—Tumors that develop in patients with Crohn's disease tend be multifocal, so field cancerization (the replacement of normal cells with non-dysplastic but tumorigenic clones) might contribute to intestinal carcinogenesis. We investigated patterns of tumor development from pretumor intestinal cell clones.

Methods—We performed genetic analyses of multiple areas of intestine from 10 patients with Crohn's disease and intestinal neoplasia. Two patients had multifocal neoplasia; longitudinal sections were collected from 3 patients. Individual crypts were microdissected and genotyped; clonal dependency analysis was used to determine the order and timing of mutations that led to tumor development.

Results—The same mutations in *KRAS*, *CDKN2A(p16)*, and *TP53* that were observed in neoplasias were also present in nontumor, nondysplastic, and dysplastic epithelium. In 2 patients, carcinogenic mutations were detected in nontumor epithelium 4 years before tumors developed. The same mutation (*TP53* p.R248W) was detected at multiple sites along the entire length of the colon from 1 patient; it was the apparent founder mutation for synchronous tumors and multiple

Address requests for reprints to: Susan Galandiuk, s0gala01@louisville.edu; fax: (502) 852-8915; or Trevor A. Graham, trevor.graham@ucsfmedctr.org.

Trevor A. Graham's present address is: Center for Evolution and Cancer, University of California, San Francisco, 2340 Sutter Street, Box 1351, San Francisco, California 94143.

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dysplas-tic areas. Disruption of *TP53*, *CDKN2A*, and *KRAS* were all seen as possible initial events in tumorigenesis; the sequence of mutations (the tumor development pathway) differed among lesions.

Conclusions—Pretumor clones can grow extensively in the intestinal epithelium of patients with Crohn's disease. Segmental resections for neoplasia in patients with Crohn's disease might therefore leave residual pretumor disease, and dysplasia might be an unreliable biomarker for cancer risk. Characterization of the behavior of pretumor clones might be used to predict the development of intestinal neoplasia.

Keywords

Inflammatory Bowel Disease; Tumor Progression; Clonal Ordering; Oncogene; CRC

Long-standing Crohn's disease (CD), an inflammatory bowel disease, is associated with an increased risk of developing intestinal cancer that is between 2 and 3 times greater than that of the healthy population.[1–4] Compared with non–inflammatory bowel disease tumors, synchronous and multifocal neoplasia occurs in as many as 30% of patients with CD with neoplastic disease.[5–7] This suggests that field cancerization, the widespread replacement of the normal cell population by a histologically nondysplastic mutant clone that is predisposed to tumor development,[8–10] could underlie tumorigenesis in CD. The identification and characterization of pretumor mutant fields in patients with CD could provide better predictors of a patient's risk of neoplasia.

The widely accepted paradigm for tumorigenesis, the somatic mutation theory, is that tumorigenesis begins with rate-limiting mutations in a key growth control gene, resulting in immediate lesion growth, and then subsequent alteration of other genes drives the evolution of subclones within the tumor, leading eventually to the development of a malignant clone.[11,12] However, the identification of field cancerization in the lung[13] and colon[14,15] and skin[16] makes it increasingly clear that tumor development can begin long before any overt tumor growth.[17] The dynamics of such pretumor clones remain singularly unknown. Determining parameters such as the clone growth rate would permit more precise estimations of a patient's risk and timescale for neoplastic development, while also revealing the basic biology of stem cell clone growth in the human intestine. Given their risk of malignancy, patients with CD are often subjected to routine endoscopic surveillance with concomitant surgery.[18–20] Thus, tissue samples are collected in some patients over many years, providing a fortuitous but uncommon means to study the longitudinal dynamics of pretumor clones in the human intestine.

The genetic pathways of tumor development in CD have not been conclusively determined; however, the etiologic similarities between CD and ulcerative colitis (UC) suggest that tumorigenesis in the 2 diseases may share genetic pathways. UC-associated neoplasia frequently shows *TP53 or KRAS* mutations,[14,21–23] whereas inactivating mutations of the adenomatous polyposis coli (*APC*) gene, found in most sporadic colorectal tumors,[24] are rarely observed.[14,22] Loss of heterozygosity (LOH) of chromosome arm 18q, possibly targeting the *SMAD4* gene, is also relatively frequent in UC cancer.[21,25]

Here, we investigate pretumor clonal dynamics in the inflamed human CD bowel. Inferring clonal dynamics in the human bowel is technically challenging, because invasive labeling studies are impractical. Instead, we used clonal ordering techniques that exploit the relative spatial location of mutations to infer the order in which they were acquired.[26,27] Rare point mutations mark clones, because 2 cells are very unlikely to bear the same point mutation unless they share a clonal origin. Longitudinally collected tissue samples allow the extent and rate of clonal expansion to be determined, indicating the relative fitness of each clone.[28]

Patients and Methods

See Supplementary Materials and Methods for full methods.

Patient and Clinical Material

Paraffin-embedded biopsy and resection specimens were obtained from 10 patients with CD-associated intestinal neoplasia (Table 1). Specimens from multiple areas of intestine were available from each patient; 3 patients had longitudinally collected samples, and 2 patients had multifocal neoplasia. The case of patient 1 is particularly enlightening because this patient had tissue collected over an 8-year period (1996–2004), during which he developed multifocal and metachronous tumors.

Mutation Screening of Cancers

Initially, DNA was needle macrodissected from each neoplastic lesion (surgical resection specimens) and screened for somatic mutations using polymerase chain reaction (PCR) sequencing at loci frequently mutated in inflammatory bowel disease cancers: *TP53* exon 5–9, *KRAS* exon 1 (codons 12–13), and *CDKN2A* ($p16^{INK4a}$) exon 2. Primer details are listed in Supplementary Table 1.

Laser Capture Microdissection

Mutations identified in the screening phase were then investigated on a crypt-by-crypt basis in multiple tissue specimens from each patient. A total of 450 crypts were microdissected from serial sections using a PALM microdissection system (Zeiss, Munich, Germany); in poorly differentiated tumors, small areas of epithelial cells were microdissected. Pathology was assessed using a serial H&E-stained slide. Following DNA extraction by digestion in PicoPure proteinase K buffer (Applied Bio-systems, Warrington, United Kingdom), DNA lysates were PCR sequenced. Tubes containing digestion buffer but no microdissection material were included as negative controls.

LOH Analysis

LOH analysis was performed on individual crypt lysates at up to 19 informative markers close to the *FHIT*, *APC*, *CDKN2A*, *SMAD4*, and *TP53* genes, respectively, using a multiplexed microsatellite PCR kit (Qiagen, Crawley, United Kingdom). LOH was then considered present if the area under one allelic peak was more than twice that of the other, after normalizing peak areas relative to constitutional DNA. Multiple informative markers located close to *APC*, *TP53*, and *CDKN2A* were available for some patients; in these cases, LOH was called if it was shown by at least 2 of the 3 available markers. Microsatellite

instability was assessed using multiplexed assay for the BAT-25 and BAT-26 mononucleotide repeats[29]; the presence of microsatellite instability in a sample precluded reliable LOH analysis. Primer details are listed in Supplementary Table 2.

Image Cytometry

Areas of approximately 1 mm^2 of epithelium were needle dissected from waxed 40-μm sections, using a serial H&E section as a guide. Image cytometry was performed using the Fairfield DNA Ploidy System (Fairfield Imaging, Kent, United Kingdom) as previously described.[14]

Immunohistochemistry

Immunohistochemistry was performed for p53 (Dako, Ely, United Kingdom), β-catenin (Transduction Labs, Oxford, United Kingdom), and lysozyme (Dako) using standard protocols.

Results

Somatic mutations in *TP53, CDKN2A,* or *KRAS were* detected in neoplastic tissue from 7 of 10 patients (Table 1). Individual histologically normal, inflamed, or dysplastic crypts, cancer glands, or small areas of poorly differentiated tumor (~500 cells) were then microdissected from multiple tissue samples from each patient with detected mutations and genotyped for the somatic mutation(s) detected in the patient's neoplasia (Supplementary Table 3).

Clonality of Tumor and Nontumor Tissue

Individual crypts from nontumor tissue were examined in the 7 of 10 patients who had a detected mutation in their neoplasia. In 5 of 7 of these patients, the mutation present within the tumor could also be detected in nontumor tissue (Figure 1 and Supplementary Table 3), suggesting in each case a clonal relationship between the tumor and nontumor mucosa.

Within nontumor tissue, the mutations found in the cancer were frequently present in both dysplastic and nondysplastic crypts, although always in areas of active disease (Figure 1 and Supplementary Table 3). Patients 3 and 4 had cancer in the small intestine, whereas patients 1, 5, and 6 had colon cancer, indicating that pretumor clone growth occurred in both intestinal compartments.

The remaining 2 patients (patients 2 and 7) had detected mutations only within the cancer and dysplasia-associated lesion or mass, respectively. Although an undetected pretumor clone may have preceded tumor growth, it is conceivable that these neoplasia followed a sporadic pattern of tumorigenesis.

Detection of Mutant Clones Many Years Before Cancer Growth and at Sites Distinct From the Cancer

Longitudinally collected samples, available from 2 informative patients (1 and 5), provided a means to study the dynamic behavior of putative pretumor clones (Figure 2 and Supplementary Figure 1). Patient 5 developed an adenocarcinoma arising within a perineal

proctectomy scar that was resected in 2008. Both the cancer and the nearby nondysplastic crypts in the inflamed resection margin contained *KRAS* c.37G>A, *TP53* c.733G>A, and *CDKN2A* mutations, and the same 3 mutations could also be detected in a proctectomy specimen collected 4 years earlier (Supplementary Table 3). However, a nearby perianal fistula tract in the earlier proctectomy specimen showed the same *KRAS* c.37G>A mutation, a different *TP53* mutation, and no *CDKN2A* mutation, indicating that *KRAS* c.37G>A was the first pretumor mutation. This second *TP53* mutation was not detected in later samples, suggesting the clone had died out (Supplementary Figure 1). Interestingly, a small bowel biopsy specimen from 2004 showed a different *KRAS* c.35G>A mutant clone in nondysplastic crypts; however, a subsequent biopsy specimen from the same area in 2006 did not contain this clone, suggesting this second *KRAS* clone had undergone only limited clonal expansion or died out.

An extensive tissue archive was available from patient 1 (Figure 2 and Supplementary Table 3). The sigmoid adenocarcinoma resected from this patient in 2000 contained a *TP53* c. 731G>A mutation throughout (Supplementary Figure 2), which was also present in morphologically nondysplastic crypts in the resection margin. Crypts from earlier rectal and sigmoid biopsy specimens (1996 and 1998) contained no detected *TP53* mutations, suggesting that the *TP53* c.731G>A mutation occurred in the 2 years before cancer growth. A follow-up biopsy around the anastomosis area did not contain the c.731G>A mutation, suggesting the resection successfully removed the mutant clone.

A second *TP53* mutant, c.775G>T, was detected in the inflamed but nondysplastic resection margin of the sigmoid cancer in 2000. The same mutation was later detected in the transverse colon mucosa resected in 2004.

A third *TP53* c.742C>T mutation was also detected in this patient, in multiple neoplastic lesions, and at multiple sites spanning the length of the colon (Figure 2). The mutation was first identified in crypts from sigmoid and rectum collected in 2001 and was not present in earlier samples, making it likely the mutation was first acquired in this area around this time. The c.742C>T mutation was again detected in sigmoid crypts collected in 2003 but not in a simultaneous rectal biopsy specimen. By 2004, the c.742C>T mutation was detected at sites along the entire length of the colon: a rectal cancer (Supplementary Figure 3), a transverse colon hyperplastic mucosa (Figure 1), a right colon adenoma, and in the nondysplastic but inflamed mucosa surrounding each of these lesions. This mutation was a founding mutation for areas of dysplasia, an adenoma, and a cancer. Immunohistochemical analysis of the patient's colectomy specimen in 2004 revealed patchy and infrequent p53 overexpression along the colon length (Figure 1), suggesting that p53 mutant clones were present only in a minority of crypts.

Noninflamed areas of terminal ileum were available from patients 3 and 4. Crypts microdissected from these nondiseased areas did not contain the mutations found in these patients' cancer and inflamed mucosa (Supplementary Figure 4).

Crypts on Either Side of the Ileal-Cecal Valve Have a Common Somatic Mutation

Individual crypts from inflamed terminal ileum tissue from patient 1 collected in 2004 contained the same *TP53* c.742C>T mutation present in this patient's colon. Separate microdissection of crypts and villi showed that the mutation was also present on the villus fed by a mutant crypt (Figure 3). Immunohistochemistry for lysozyme staining highlighted the presence of Paneth cells within mutated crypts (Figure 3).

Interchangeable Order of Mutations in Tumorigenesis

The order of mutations in tumorigenesis differed between tumors. Mutation order was inferred from the relative spatial localization of mutants; genetic heterogeneity was a hallmark of most tumors. In all 3 polypoid lesions detected in patient 1, a *TP53* point mutation was present throughout the lesion and within the resection margins, suggesting *TP53* was the founder mutation for each lesion. Tumor development involved sequential 17p, 9p and 18q LOH, and aneuploidy (Figure 4 and Supplementary Figure 3). The sigmoid cancer resected in patient 1 in 2000 also showed widespread 5q LOH and nuclear β-catenin (Supplementary Figure 2), implicating *APC* in early tumorigenesis of this lesion.

Biallelic mutation of *TP53*, presumed on the detection of both a *TP53* point mutation and LOH, was not requisite for tumor growth; the ascending colon adenoma in patient 1 in 2004 had a *TP53* point mutation throughout the adenoma but only a subclone had 17p LOH; furthermore, biallelic *TP53* mutations were infrequently observed in nondysplastic crypts in this patient (Supplementary Table 3). Similarly, a putative biallelic *TP53* mutation was observed in the dysplastic margins of the cancers from both patients 5 and 6.

The presence of a *CDKN2A* mutation in both tumor and nondysplastic tissue in patient 3 implicates *CDKN2A* disruption in the initiation of this patient's tumor; tumor progression involved a subsequent *KRAS* mutation and the development of microsatellite instability (Figure 4). The initial mutation in patient 5 was in KRAS, with later *CDKN2A* and *TP53* mutations implicated in subsequent tumor development.

Discussion

The genetic and histologic mechanisms driving the development of CD-associated cancers have not been conclusively determined. The data presented herein are strong evidence that field cancerization, the replacement of the normal epithelium with a protumorigenic clone,[8,9] before any dysplastic histologic change contributes to carcinogenesis in patients with CD. In 5 of 7 informative patients with neoplasia, the same point mutation in *KRAS*, *CDKN2A*, or *TP53* could be detected within the tumor, neighboring resection margins that showed active disease, and even in more distant diseased areas, strongly suggesting that this mutant tissue was clonal in origin.

In 1 patient, a *TP53* c.742C>T mutation was first detected focally in the sigmoid colon and then 4 years later at sites along the entire colon length. These data suggest that the mutant clone had arisen in the left colon and appeared to have spread both proximally to the right colon and distally to the rectum within 4 years (Figure 2), suggesting pretumor growth can occur at a substantial scale and rate. This conclusion rests on the strength of the *TP53* c.

742C>T point mutation to uniquely identify a clonal population of cells in this patient. If this particular mutation occurred at high frequency, it is conceivable that 2 unrelated cells could independently develop the mutation; if this were true, then the data suggest pretumor clone growth of more restricted spatial extent. The International Agency for Research on Cancer p53 mutation database[30] suggests that the c.742C>T mutation comprises approximately 7% of the total detected somatic *TP53* mutations in the human intestine. Given 2 crypts that both have a *TP53* mutation, the odds that crypts will both have the c.742C>T mutation are small (approximately 200:1). This calculation assumes that the distribution of *TP53* mutations selected in the CD bowel mirrors that reported in the International Agency for Research on Cancer database for the intestine at large. However, given that other gastrointestinal cancers arising in an inflammatory environment show a broad range of *TP53* mutations, this is likely a reasonable assumption.[14,31–33] The notion that the *TP53* c.742C>T mutation marks a single clone is further supported by LOH data; both the rectal and ascending colon lesions in patient 1, which were clonal for the *TP53* c.742C>T point mutation, showed loss of the same alleles of markers on chromosome arms 9p, 18q, and 17p. Because the same allele of 17p was consistently lost around the entire colon, this suggests that the universally retained allele bore the c.742C>T point mutation. The odds of finding 2 cells, which both have LOH at these 3 loci and have lost the same alleles, are 1:8; the odds of 2 mutated cells both bearing these same patterns of LOH and the c.742C>T point mutation are at most 1:1600. This probability must also be considered in light of the presumably very small likelihood of a crypt acquiring a fixed genetic lesion at all. Therefore, it is likely that the widely dispersed *TP53* c.742C>T mutant cells, frequently marked by an additional 3 genetic lesions, in patient 1 comprised a single clone. Furthermore, evidence of long-range clone spread in this patient is clearer still when the *TP53* c.775G>T mutant is considered. This mutation is reported to be relatively rare, comprising approximately 0.1% of the reported somatic *TP53* variants in the International Agency for Research on Cancer database, and so the odds of this mutant arising more than once in the bowel are minimal. The *TP53* c.775G>T mutation was detected in the descending colon at baseline and the transverse colon 4 years later, indicating extensive clone migration during this time.

How does a mutant clone grow in the CD intestine? Crypt fission is the primary mechanism of clonal expansion in the intestinal epithelium.[34,35] Although fission is rare in the normal intestine, patients with colitis have an elevated fission rate,[36] and tumorigenic mutations may further increase the crypt division rate. Chronic inflammation, resulting in cycles of crypt atrophy and mucosal healing by crypt fission, likely provides a major growth stimulus; indeed, mutant clones were only identified in areas of active disease. Despite this, it is unlikely that a mutant clone could sustain exponential growth,[37] raising the possibility that extensive clonal expansion may occur through a noncanonical mechanism such as stem cell migration between crypts, perhaps analogous to the migration between niches observed in the Drosophila ovary.[38]

p53 and p16 mutants were observed to have undergone extensive clonal expansion in the colitic bowel, indicating that these mutated clones have a selective advantage in the CD intestine. Mutations that impair the ability of p53 and p16 to regulate the apoptosis and

senescence responses presumably provide a survival advantage, because such mutant clones are likely more able to withstand the inflammatory stress of the colitic bowel. Specific clone fitness may be determined by the mutation type; indeed, the c.742C>T, p.R248W mutant, which underwent the most extensive clonal expansion, is associated with severe down-regulation of p53 activity.[30,39] Differential effects of each mutation may also regulate the ability of a mutant cell to compete with wild-type or other mutant cells for a place in the bowel.[40] Further selective advantage for *TP53* mutants may be due to their tolerance of genetic instability,[41,42] seemingly an early event in colitis-associated cancers.[43] Supporting this hypothesis, an aneuploid population was observed in transverse colon nondysplastic but p53-mutant tissue in patient 1 (Supplementary Table 3 and Supplementary Figure 5).

Notably, mutations were only detected in diseased tissue; consequently, we would suggest that there is interdependence between the persistence of mutant clones and inflammatory disease. This relationship gives further credence to the suggestion that tumor suppressor gene mutations could confer a survival advantage in the inflamed bowel; indeed, 4 of the 5 patients who had identified field cancerization were also recorded as having long-standing active CD. Further, it is tempting to speculate that persistent inflammation may be necessary for significant clone growth. Fission rates are markedly increased in inflamed mucosa,[36] and potentially this is the direct cause of clone spread. Future analyses of isolated “skip lesions” and their histologically normal margins, and also of pathology archives that fully catalog nondiseased bowel, will provide a means to fully investigate any relation between active disease and clone spread. Our data are insufficient to investigate any potential relationship between mucosal injury from an endoscopic biopsy and clone growth.

In one patient, the same *TP53* mutation was detected in crypts on both sides of the ileal-cecal valve. These data therefore suggest that a colon-derived cell can cross into the small intestine and form functional crypt-villus units. The strength of this conclusion depends on the reliability of the *TP53* mutation as a clone marker; its merits have been discussed previously. There are a number of possible mechanisms for this apparent cell migration. First, an impaired ileocecal valve, perhaps compromised by inflammation, could permit retrograde clone expansion. Second, the relatively common fissures in patients with CD[44] could provide a migratory route for a cryptogenic cell. Third, it is conceivable that endoscopy could seed colon-derived cryptogenic cells into the small intestine; denuded epithelium in areas of active disease may provide a fertile ground for migrating cells. Once in the small intestine, mesenchymal signaling may then induce the progeny of the colon-derived stem cell(s) to follow a small intestinal pattern of differentiation.[45,46]

The clinical implications of our findings could be considerable. First, we have shown that tumorigenic mutations may be present in large sections of morphologically nondysplastic mucosa. This questions the adequacy of dysplasia as a biomarker for neoplasia risk, because nondysplastic crypts can carry what may prove to be a biologically significant mutation burden. Molecular profiling of the nondysplastic epithelium could potentially better identify patients at risk for neoplasia, with the detection of particular mutants predicting enhanced risk. A controlled study comparing the mutant-clone burden and dynamics between patients with CD cancer and patients without cancer over time is required. Second, it questions the adequacy of performing endoscopic or limited resections for colonic neoplasms in patients

with CD, but close surveillance in patients with long-standing Crohn's colitis is still needed until more is understood about the process. It is conceivable that patients who have developed a prolific mutant clone may be best treated by colectomy rather than limited resection, whereas a less prolific clone could be dealt with by localized treatment, although an assessment of both the efficacy and cost-effectiveness of these suggestions would need to be performed. Definite conclusions here are prohibited by the absence of data from patients who never acquired cancers; this is a limitation of our study. The remarkable motility of pretumor clones may be a hallmark of other inflammation-associated cancers such as hepatocellular carcinoma, cholangiocarcinoma, and Barrett's-associated adenocarcinoma, highlighting the need for further investigation into the dynamics of pretumor clones to understand patterns of disease occurrence.

We have shown frequent and occasionally very extensive field cancerization in the chronically inflamed bowel of a few patients with CD. These data provide a precedent for further study of pretumor clones as biomarkers in inflammatory diseases. Our observation of nondysplastic crypts harboring tumorigenic clones questions the utility of dysplasia as the sole biomarker of neoplastic risk. Foremost, we have highlighted that pretumor clonal dynamics can contribute to patterns of tumor occurrence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used in this paper

CD	Crohn's disease
LOH	loss of heterozygosity
PCR	polymerase chain reaction
UC	ulcerative colitis

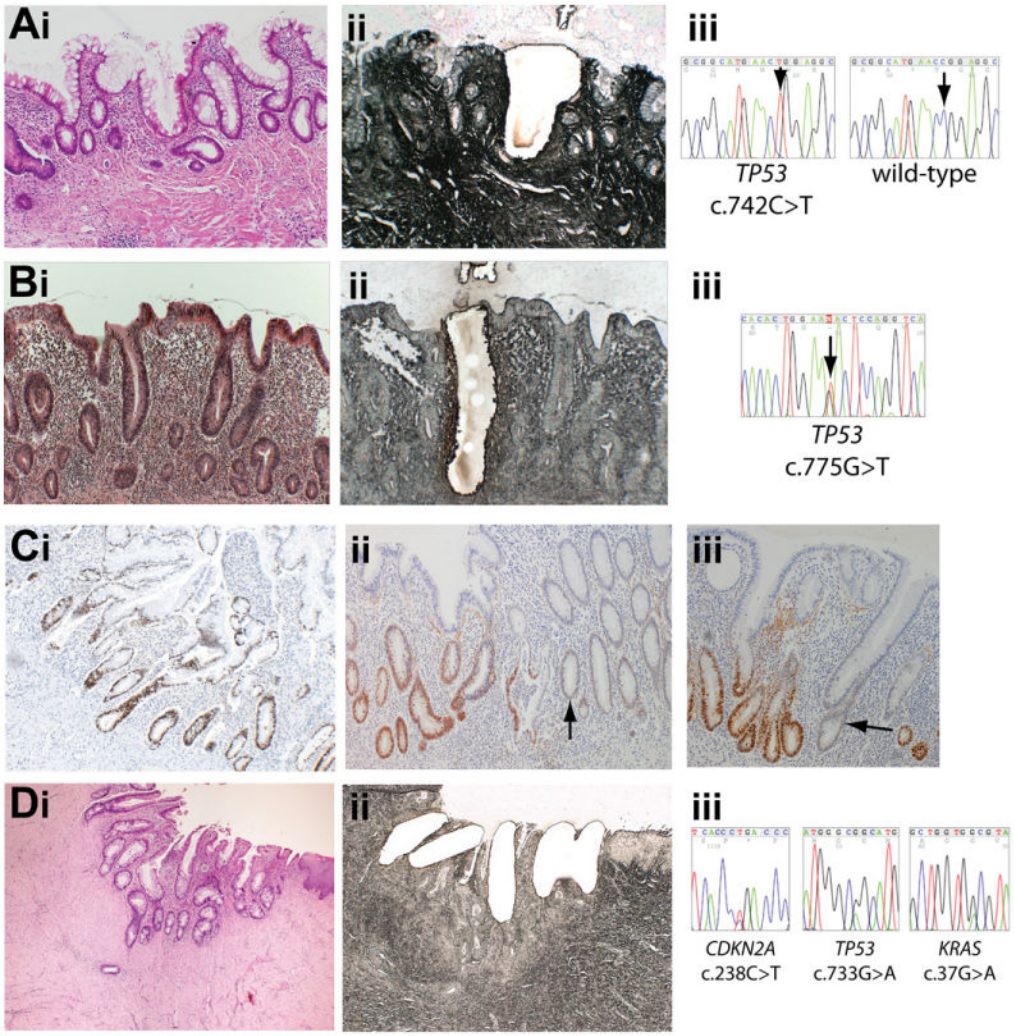

Figure 1.

Mutations present in nontumor tissue. (*A*) (*i*) H&E stain (original magnification 100×) of nondysplastic (hyperplastic) mucosa showing crypt distortion with increased inflammatory cells and intraepithelial neutrophils in the transverse colon in patient 1 in 2004 and (*ii*) serial methylene green–stained PALM laser capture slide showing microdissected crypt. (*iii*) Sequencing shows the crypt contains a *TP53* c.742C>T mutation. (*B*) (*i*) H&E stain (original magnification 100×) of nondysplastic (hyperplastic) mucosa with a marked increase in the inflammatory cells in the lamina propria and cryptitis in the resection margin of the sigmoid cancer resected from patient 1 in 2000 with (*ii*) serial PALM slide showing microdissection. (*iii*) Sequencing shows the crypt contains a *TP53* c.775G>T mutation. (*C*) p53 immunohistochemistry on a nondysplastic colon resection specimen from patient 1 (original magnification 100×). (*i*) Patches of crypts with nuclear accumulation of p53 protein were observed, frequently demarked by odd p53-negative or low-expressing crypts (*ii* and *iii*) (*arrows*). (*D*) (*i*) H&E stain (original magnification 40×) of inflammatory atypia within cells in a perianal fistula from patient 5 four years before tumor growth. (*ii*) Laser capture slide (original magnification 100×). (*iii*) *TP53*, *CDKN2A*, and *KRAS* mutations in each crypt.

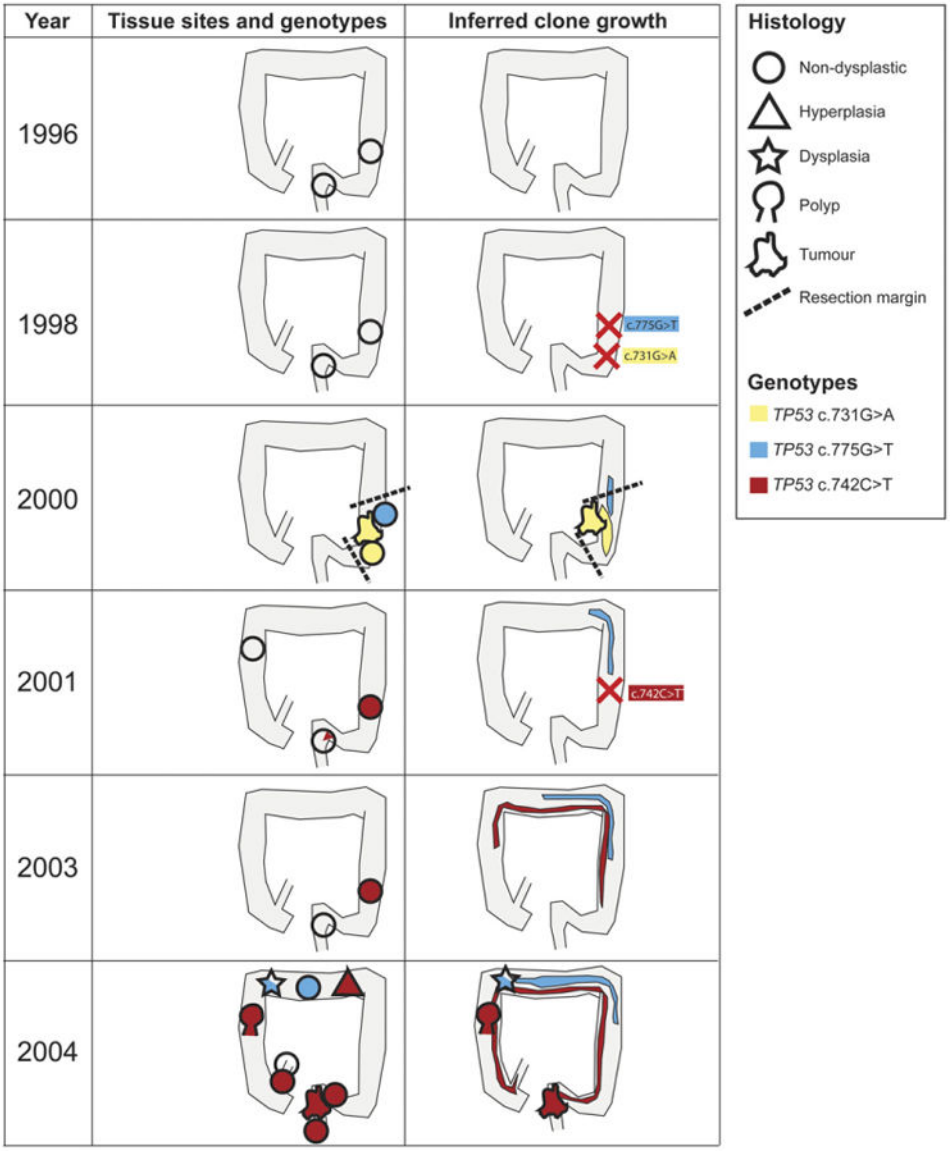

Figure 2.

Longitudinal analysis showing clone spread in patient 1. Tissue collected before 2000 contained no widespread detected genetic abnormalities. A c.731G>A *TP53* mutation was first detected throughout a sigmoid cancer in 2000 and within the resection margins, but not in later samples, suggesting the mutant arose in the sigmoid after the biopsy in 1998 and was completely removed by the cancer resection. A second c.775G>T *TP53* was also found in the resection margin of the sigmoid cancer. This second clone had spread to the transverse colon by 2004. A third *TP53* c.742C>T mutation was first detected in sigmoid and rectal biopsy specimens from 2001. By 2004, the mutant *TP53* c.742C>T clone was present in every major segment of the colon and also in the terminal ileum and in multifocal neoplasia. *Shapes* indicate the predominant pathological diagnosis at the site, and *color* indicates the predominant *TP53* genotype (see *key*).

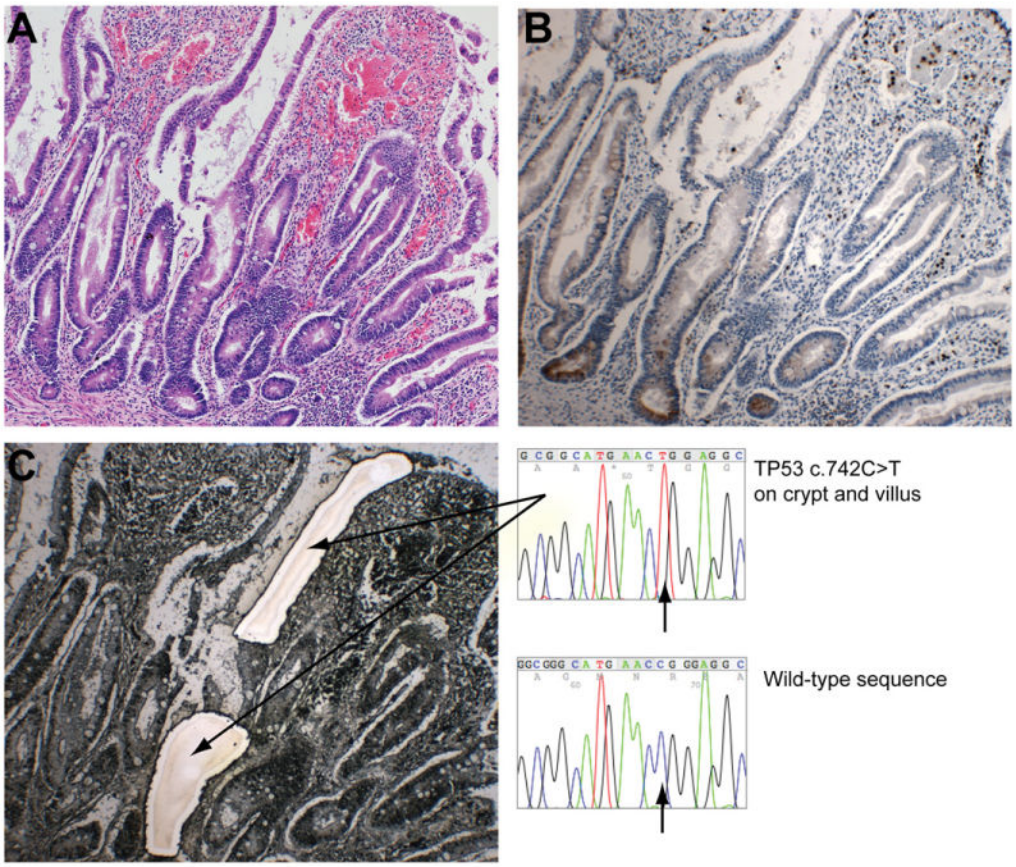

Figure 3.

Presence of the same *TP53* mutation in functional small intestinal and colon crypts. (*A*) H&E section showing inflamed terminal ileum mucosa with crypt/villous distortion and an increase in inflammatory cells and intraepithelial neutrophils. (*B*) Serial section stained for lysozyme indicating the presence of functional Paneth cells at the small intestinal crypt base. (*C*) Serial laser capture slide stained with methylene green showing separately microdissected crypt and villus. Genotyping revealed the same *TP53* c.742C>T was present in both the crypt and villus. This same mutation was found in colon tissue collected at the same and previous times. Original magnification 100× for all micrographs.

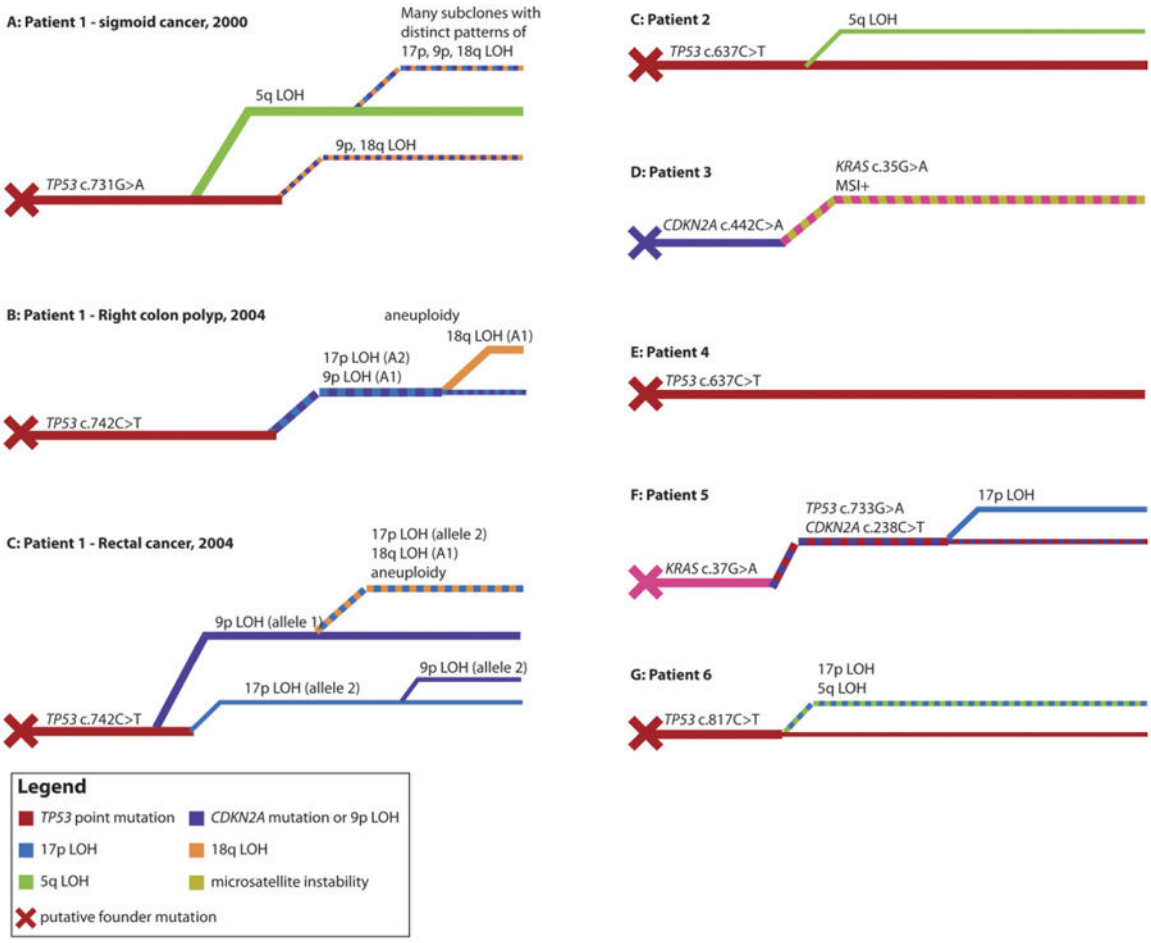

Figure 4.

Phylogenetic trees of neoplasia development. (*A*) Phylogenetic tree of sigmoid cancer resected in patient 1 in 2000. Tumor growth was preceded by the acquisition of a *TP53* c.731G>A mutation, because this mutation was found in the cancer and its resection margin. A clone with 5q LOH was dominant in the cancer, and a single crypt showed 9p and 18q LOH but no 5q LOH. Numerous small subclones with distinct patterns of 9p, 18q, and 17p LOH were found within the 5q subclone. This frequent LOH could be attributable to the cancer containing an aneuploid clone, which was not tested for in this cancer. (*B*) Phylogenetic tree of the ascending colon adenoma resected in patient 1 in 2004. The cancer developed from the preneoplastic clone distinguished by the *TP53* c.742C>T mutation, and subsequent carcinogenesis involved the sequential acquisition of 17p, 9p, and then 18q LOH. Unlike the previous cancer in patient 1, no 5q LOH was detected in the lesion in 2004. (*C–G*) Phylogenetic trees for tumors from patients 1–6. Most lesions were initiated by a *TP53* mutant clone, with the exceptions of patient 3, where the putative founder clone was a *CDKN2A* (p16) mutant, and patient 5, where the founder clone was a *KRAS* mutant. The inferred sequence of subsequent mutations and LOH events was different in every lesion. *Colors* indicate genotypes (see *key*), and line thicknesses are indicative of relative clone abundance.

Table 1

Patient Details

Patient	Age (*y*) CD diagnosed	Age (*y*) neoplasm diagnosed	Family history of inflammatory bowel disease	Family history of colorectal cancer	Location of CD	Immunomodulator use	No. of neoplasms	Location of neoplasm	Type of neoplasm(s)	Mutant genes detected	TP53	Kras	p16	Tissues examined over time
1	65	73	No	No	Pancolonic and terminal ileum	No	3	Sigmoid Sigmoid Rectum	Moderately differentiated adenocarcinoma High-grade dysplasia Poorly differentiated adenocarcinoma	*TP53*	Exon 7 c. 731G>A G244D Exon 7 c. 742C>T R248W Exon 7 c. 742C>T R248W	None	None	1996 1998 2000 2001 2003 2004
2	39	39	No	Yes	Ileocolic	No	1	Sigmoid	Moderately differentiated adenocarcinoma	*TP53*	Exon 6 c. 637C>T R213*	None	None	No
3	44	70	No	No	Small bowel and ileocolic	Yes, azathioprine	1	Jejunum	Moderately differentiated colloid adenocarcinoma	*Kras p16*	None	c.35G>A G12D	c.442C>A A148T	No
4	29	30	Yes	Yes	Ileocolic	No	1	Right colon	Moderately differentiated adenocarcinoma	*TP53*	Exon 6 c. 637C>T R213*	None	None	No
5	37	65	No	No	Pancolonic terminal ileum, jejunum perianal	Yes, azathioprine	1	Perineal wound in fistula tract	Well-differentiated mucinous adenocarcinoma	*TP53 Kras p16*	Exon 7 c. 733G>A G245S	c.37G>A G13D	c.238C>T R80*	2004 2006 2008
6	37	52	Yes	No	Colonic	No	1	Rectal	Moderately differentiated adenocarcinoma	*TP53*	Exon 8 c. 817C>T R273C	None	None	No
7	71	86	No	No	Colonic	No	2	Transverse colon	Adenomas (DALM)	*Kras*	None	c.35G>A G12D	None	2009 2010
8	10	32	Yes	Yes	Ileocolic	No	1	Ileum	Poorly differentiated mucinous adenocarcinoma	None	None	None	None	No
9	45	46	No	No	Ileocolic perianal	No	1	Right colon	Poorly differentiated adenocarcinoma	None	None	None	None	No
10	78	79	No	No	Ileocolic	No	1	Right colon	Mucinous moderately differentiated adenocarcinoma	None	None	None	None	No

DALM, dysplasia-associated lesion or mass as adenoma was in area of colitis.