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Genomic Alterations Observed in Colitis-associated Cancers are Distinct from Those Found in Sporadic Colorectal Cancers and Vary by Type of Inflammatory Bowel Disease

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Abstract

Background & Aims—Patients with inflammatory bowel diseases such as Crohn's disease (CD) or ulcerative colitis (UC) are at increased risk for small bowel or colorectal cancers (colitis-associated cancers, CACs). We compared the spectrum of genomic alterations in CACs with those of sporadic colorectal cancers (CRCs) and investigated differences between CACs from patients with CD vs UC.

Methods—We studied tumor tissues from patients with CACs, treated at Memorial Sloan Kettering Cancer Center or Weill Cornell Medical College from 2003 through 2015. We performed hybrid capture based next-generation sequencing analysis of over 300 cancer-related genes to comprehensively characterize genomic alterations.

Results—We performed genomic analyses of 47 CACs (from 29 patients with UC and 18 with CD; 43 primary tumors and 4 metastases). Primary tumors developed in the ileum (n=2), right colon (n=18), left colon (n=6) and rectosigmoid or rectum (n=21). We found genomic alterations in *TP53*, *IDH1*, and *MYC* to be significantly more frequent, and mutations in *APC* to be significantly less frequent, than those reported in sporadic CRCs by The Cancer Genome Atlas or Foundation Medicine. We identified genomic alterations that might be targeted by a therapeutic agent in 17/47 (36%) of CACs. These included the mutation encoding IDH1 R132; amplification of FGFR1, FGFR2, and ERBB2; and mutations encoding BRAF V600E and an EML4-ALK fusion protein. Alterations in *IDH1* and *APC* were significantly more common in CACs from patients with CD than UC.

Conclusions—In an analysis of CACs from 47 patients, we found significant differences in the spectrum of genomic alterations in CACs compared to sporadic CRCs. We observed a high frequency of *IDH1* R132 mutations in patients with CD but not UC, as well as a high frequency of *MYC* amplification in CACs. Many genetic alterations observed in CACs could serve as therapeutic targets.

Keywords

IBD; inflammatory bowel disease; bowel cancer; cancer of the ileum

Introduction

Patients with inflammatory bowel disease (IBD) are at a substantially increased risk for cancers of the colon/rectum or (for Crohn's Disease) the small bowel, and the risk increases the longer IBD is active[1]. Colitis-associated cancers (CAC) are an especially feared complication of IBD, as they are frequently diagnosed at an advanced stage, with locally advanced or metastatic disease. Unlike more common sporadic colorectal cancers (CRC) that arise from polyps, it may be difficult to identify pre-cancerous dysplastic lesions or early CAC in patients with IBD. Their flat appearance makes the total extent of the pre-cancerous area difficult to determine and to remove endoscopically. CAC commonly present with multifocal tumors and signet ring cell histology, features associated with a worse prognosis[2-6].

The biologic changes that underlie the development of CAC are not well understood, but current data suggest that the genetic events leading to the initiation of sporadic CRC are different than those leading to CAC. Chronic inflammation is assumed to lead to genomic changes that increase the risk of CAC, and animal models suggest that both the initiation and the progression of colonic neoplasia can be exacerbated or expedited by an inflammatory insult[7, 8]. *TP53* mutations are an early event in CAC and are often detected in non-dysplastic areas or in dysplasia in patients with IBD[9, 10], while *TP53* mutations are considered late events in sporadic CRC. In sporadic colorectal adenocarcinoma, WNT pathway activation, particularly through *APC* mutational inactivation, is often the initiating event[11, 12]. It is not yet clear if subsequent genomic alterations in CAC are similar or different from those occurring in sporadic CRC. This is of importance as new therapies are frequently designed to affect specific alterations, and potentially targetable alterations in CAC have not been clearly defined.

After a genomic alterations analysis revealed an unexpected EML4-ALK fusion protein in a tumor specimen from a patient with CAC, we set out to more comprehensively characterize the mutational landscape of CAC. In this study, we use hybrid capture-based next-generation sequencing (NGS) to define recurrent or targetable genomic events in CAC from patients with both ulcerative colitis (UC) or Crohn's disease (CD) and compare the spectrum of genomic alterations (GA) in CAC with those occurring in sporadic CRC.

Methods

Samples

We queried the pathology databases to identify all cases of bowel cancer seen at Memorial Sloan Kettering Cancer Center (MSKCC) and Weill Cornell Medical College- New York Presbyterian Hospital (WCMC) between 2003 and 2015 in which colitis as a clinical factor was indicated in the report. The medical records were reviewed and tissue from all potential cases of CAC was retrieved. Pathology slides from all cases were reviewed by a pathologist (L.T., R.K.Y.) to confirm evidence of colitis and carcinoma, and the medical records were reviewed by a medical oncologist (R.Y., M.A.S., D.K.) for the clinical history of IBD. The pathologist selected the appropriate tissue blocks for DNA extraction. This study was exempt from IRB review after institutional MSK IRB review (IRB waiver WA0143-14).

Genomic analysis

Samples were analyzed using hybrid capture NGS, either with FoundationOne (42 cases) or the MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets) assay (5 cases). MSK-IMPACT testing was used in the five cases with small amounts of tumor DNA. DNA was extracted from formalin fixed paraffin embedded samples by digestion in a proteinase K buffer followed by purification with Promega Maxwell 16 Tissue LEV DNA kit (for FoundationOne) or using the Qiagen DNeasy Blood & Tissue Kit modified for deparaffinisation (for MSK-IMPACT). The DNA was then subjected to hybridization capture of the entire coding sequence of a large number of cancer-related genes, 315 genes in the FoundationOne assay and 341 genes with MSK-IMPACT. As described previously[13], in the FoundationOne assay, base substitution detection was performed using a Bayesian

methodology, which allows detection of novel somatic mutations at low mutant allele frequency. *De novo* local assembly in each targeted exon was applied, using the de-Bruijn approach to detect small insertions and deletions (indels). Amplifications and homozygous deletions of genes were detected by comparing complete chromosomal copy number maps to process-matched normal control samples as reference. Finally gene fusions and rearrangements were identified by analyzing chimeric read pairs from mapping. The FoundationOne assay was run on the Illumina HiSeq 2500 platform. Target specific-probes for hybrid selection in MSK-IMPACT were designed as previously described[14-16]. MSK-IMPACT is a targeted exome capture assay with ultradeep sequencing coverage using Illumina HiSeq 2000. All samples were sequenced to high, uniform coverage (>500× median coverage). All classes of GA including substitution, indels, copy number alteration, and rearrangement were determined. See full list of genes analyzed in the Supplementary Tables 1 and 2.

To identify recurrently altered pathways, we systematically analyzed all altered genes and looked for alterations in signaling pathways previously associated with colorectal cancer and other cancer types[17]. We considered mutations and focal copy number alterations (amplifications and deletions). We filtered out putative germline variants (in samples without matched normals) and mutations of unknown significance (missense mutations that were not recurrent or had not previously been described). Amplifications and missense mutations in recurrent positions in oncogenes were considered activating, and deletions, truncating mutations as well as recurrent missense mutations in tumor suppressor genes were considered inactivating. For TP53, truncating mutations and all missense mutations in the DNA binding domain were considered to be oncogenic, and some of the missense mutations are potentially gain-of-function[18].

Statistical analysis

The frequency of GA in CAC was compared to The Cancer Genome Atlas - CRC (TCGA-CRC)[17] and to a second dataset of advanced CRC cases analyzed by Foundation Medicine[19]. We also compared the frequency of GA in CD-associated CAC and UC-associated CAC. Comparisons were performed using Fisher's exact test. Significance was set at a p value <0.05.

Results

Patient characteristics

We identified 47 cases of carcinoma arising in the small or large bowel confirmed as being associated with IBD at MSKCC or WCMC, including 29 cases associated with UC and 18 cases associated with CD. Clinicopathologic characteristics of the cases are summarized in Table 1. Analyzed specimens consisted of tumor tissue from 43 primary cancers and, in 4 cases, from a metastatic site. The median patient age at diagnosis was 55 years (range 22-79), and approximately half of the patients were men. The primary tumor sites were ileum (2), right colon (18), left colon (6), and rectosigmoid/rectum (21).

Genomic profile of CAC

Overall, 6.2 GA per tumor were detected (range 1-19). We defined a recurrent alteration in CAC as one in which the gene was altered in at least 10% of cases (Figure 1). GA in *TP53*, the most commonly altered gene, occurred in 42 cases (89%). Most of the *TP53* mutations occurred in the DNA-binding domain (Supplementary Figure 1) and largely consisted of point mutations. Of the latter, 29 cases harbored missense mutations. Supplementary Table 3 lists the *TP53* alterations, with case order corresponding to the oncoprint in Figure 1. Sixteen cases had mutations reported to confer p53 oncogenic gain-of-function (GOF)[20] (C176F, V173L, R175H, R273H, R248Q in five samples, R248W in three samples, P278S, and R282W in three samples).

WNT pathway alterations in CAC were less frequent than in sporadic CRC; *APC* alterations were seen in 21% of cases, *CTNNB1* alterations in two cases, and *RNF43* mutations in three cases. *KRAS* mutations or amplification occurred in 40% of cases. *SMAD4* alterations were seen in 17% of cases, and *GNAS* alterations in 13% of cases. *IDH1* hotspot R132H/C/L mutations were detected in five cases of CAC, all associated with CD.

The most common copy number alteration was *MYC* amplification, in 26% of cases, affecting both CD- and UC-associated cases. Recurrent alterations in cell cycle genes, consisting primarily of copy number alterations of cyclin D (*CCND1, CCND2, CCND3*), cyclin dependent kinases 4, 6, and 8, and cyclin E, occurred in 16 cases (35%). Additionally, a segment of chromosome 20, containing *SRC, BCL2L1, TOP1*, and *ZNF217*, was recurrently amplified in four cases (data not shown).

Key GA associated with early onset IBD were not identified. Several cases had alterations in the Notch gene family. Sixteen cases (34%) had mutations involving *NOTCH1, NOTCH2, NOTCH3*, or *NOTCH4*, including a truncating mutation in one case. Of the alterations in the Notch gene family, seven cases had mutations previously described in at least one other tumor specimen in the Catalogue of Somatic Mutations in Cancer (COSMIC) (data not shown).

GA occurring in CAC differ significantly from GA in sporadic CRC

The spectrum of GA we identified suggests a different biology for CAC than that of sporadic CRC. We compared the incidence of recurrent GA found in CAC with their frequency in The Cancer Genome Atlas CRC (TCGA-CRC)[17] and in a large Foundation Medicine dataset of 3117 patients with CRC[19] (Figure 2). As our CAC cohort consisted predominantly of operable primary tumors (91%) (Table 1), we chose to use the TCGA-CRC data as one of the comparison populations; TCGA-CRC consists of surgical specimens from newly diagnosed colon and rectal tumors with predominantly resectable disease. We also compared the spectrum of CAC GA with that of the Foundation Medicine database (FM-CRC); we recognize that sporadic CRC tumor samples studied using FoundationOne assay are largely from patients with more advanced disease, frequently metastatic.

APC alterations were much less common in our CAC cases than in the TCGA-CRC or FM-CRC ($p<0.001$). Genomic alterations in *TP53* were nearly universal in CAC compared to about half of the cases in TCGA-CRC ($p<0.001$) and about three-quarters of Foundation

Medicine cases of advanced CRC (p=0.02). *MYC* amplification occurred in 26% of CAC compared to 4% of TCGA-CRC and 9% of advanced FM-CRC (p<0.01).

IDH1 mutations seen in 11% of CAC (and only in CD CAC) are extremely rare in sporadic CRC, with 1% mutation frequency in TCGA-CRC and only 0.6% in FM-CRC[19]. The *IDH1* mutations identified in CAC were at the R132 hotspot, producing a mutant protein that could potentially be targeted by selective IDH1 inhibitors that are currently in clinical trials.

An understanding of genomic differences between CAC and sporadic CRC may help in the clinical assessment of bowel cancers developing in patients with IBD. In an additional patient with a long prior history of CD that had been quiescent for 10 years, who underwent ileocolectomy for a rectal tumor, review of his pathologic specimen showed no evidence of active colitis (Figure 3). Genomic analysis of the resected rectal tumor (not included in our 47 patients with clear CAC) showed no *TP53* mutations, but identified *APC* and *KRAS* mutations, a genomic profile more consistent with sporadic CRC. This patient's case suggests that genomic analysis of CAC may help distinguish bowel cancer developing from underlying colitis-inflammation versus sporadic tumorigenesis.

Differences in the GA signature for CD-associated and UC-associated CAC

The GA identified in the CAC cases suggest that different genes are recurrently altered in CD-associated CAC when compared with UC-associated CAC (Figures 1 and 2). *APC* alterations were significantly more common in the CD-associated CAC (CD 39% versus UC 10%, p=0.02). Activating alterations in *KRAS* appeared more common in the UC-associated CAC, but the difference did not reach statistical significance (CD 33% versus UC 45%, p=0.5). *TP53* alterations predicted to cause p53 loss-of-function (deletions, frameshifts, and stop codons, expected to truncate p53) were more common in UC-associated CAC compared to CD-associated CAC, but again did not reach statistical significance (UC 32% versus CD 12%, p=0.16) (Supplementary Table 3).

Most striking, all the *IDH1* mutations identified were in CD-associated CAC. The *IDH1* mutations that we identified in CAC occurred exclusively in the CD cases and were seen in 5/18 CD cases and zero in 29 UC cases (CD 28% versus UC 0%, p<0.01). Notably, in one case where a patient carried a long-standing diagnosis of UC, but was found at surgery to have histopathologic features more consistent with CD, including relative sparing of the distal segment of colon and the presence of well-formed submucosal granulomas and transmural lymphoid aggregates, an *IDH1* mutation was identified (Figure 4). This suggests that the genomic profile of CAC may help clarify cases developing in the setting of UC from those developing in the setting of CD, as separating UC from CD can be difficult in a subset of IBD.

Potentially actionable alterations in CAC

Excluding *TP53* alterations, potentially actionable GA were identified in 17/47 (36%) of CAC (Figure 5). Activating alterations were commonly seen in receptor tyrosine kinase (RTK) signaling, including the fibroblast growth factor receptor (FGFR) pathway that was altered in 8 patients, *ERBB2* amplification in 2 patients, *ERBB2* S310F, *EML4-ALK* fusion, and *PDGFRA* T134M. One patient had an *FGFR2-TACC2* fusion and *FGFR2* amplification.

One patient had a *BRAF* V600E mutation and *TSC2* truncation, two alterations in mitogenic signaling pathways that are potentially targetable with RAF inhibitors and rapalogs, respectively. As noted above, potentially targetable *IDH1* hotspot R132H/C/L mutations were detected in five cases of CAC. IDH1/2 inhibitors are now in clinical trials, as are agents targeting the fibroblast growth factor (FGF) pathway. Drugs targeting HER2, EML4-ALK, and V600E BRAF are approved for other indications. Figure 5 also shows the overlap in these GA with RAS activation and indicates that the GA in receptor tyrosine kinase signaling were largely mutually exclusive of *KRAS* activation. *KRAS* and *IDH1* mutations were also not co-incident.

Activating alterations were also seen in insulin growth factor receptor signaling, including *IRS2* amplification in 3 cases and *IGF1R* amplification in 1 case (data not shown). Activating mutations in the gene encoding the catalytic subunit of phosphatidyl inositol 3-kinase (PI3K), *PIK3CA*, occurred in three cases. These GA may be targeted, alone or in combination regimens as antibodies against the insulin growth factor 1 receptor and small molecule inhibitors of PI3K are in clinical development.

Figure 6 summarizes pathways altered in UC- and CD-associated CAC, integrating data on gene mutations and copy number alterations. Alterations in the p53 pathway were seen in 100% of CD-associated CAC and 83% of UC-associated CAC. Activation of RTK/RAS signaling was common, occurring in 72% of CD-associated CAC and 57% of UC-associated cases. *FGFR2* amplification and translocations and *EGFR* activation were more common in CD-associated cases. Alterations in PI3K/mTOR signaling occurred in 22% of CD-associated CAC and 7% of UC-associated cases. Cell cycle alterations were seen in 17% of CD-associated CAC and 24% of UC-associated cases. Alterations in WNT signaling, TGFβ, and MYC were seen in about half of the CAC, with inactivation of APC more common in CD-associated CAC and *MYC* amplification affecting both CD and UC-associated cases, as noted above.

Correlations of GA in CAC with clinicopathologic features

Ten patients in our study had IBD for less than ten years at the time of diagnosis of CAC. Six of these ten patients had activation of the mitogen activated protein kinase pathway (*KRAS* mutations in 4 cases, *KRAS* amplification in 1 case, *BRAF* V600E in 1 case) and one additional case had a *PIK3CA* activating mutation, raising the possibility that secondary mitogenic alterations in this pathway, on a background of *TP53* mutation in colitis, may accelerate tumorigenesis.

Further, preclinical data suggest that *TP53* mutations with oncogenic GOF are associated with more rapid development of CAC in mouse models. We evaluated the spectrum of *TP53* mutations by duration of IBD and stage of CAC at diagnosis (Supplementary Table 3). Putative GOF *TP53* mutations were identified in five of the 10 cases occurring in patients with IBD duration less than 10 years compared to 10 of 36 cases in patients with IBD of longer duration (p=0.26). There was no clear association between the type of *TP53* alteration and the stage at diagnosis (Supplementary Table 3).

APC mutation and *MYC* amplification status were not significantly associated with duration of IBD, stage of CAC, or site of primary tumor (Supplementary Table 4). Rectal/rectosigmoid primary site was more common in patients whose tumors did not harbor an *IDH1* mutation (50% versus 0%, p=0.06); *IDH1* mutation status was not associated with duration of IBD or stage of CAC (Supplementary Table 4).

We reviewed the treatment history for IBD to address the potential relationship of IBD severity and IBD treatment history with the GA in CAC. Most patients were referred for oncologic evaluation to our hospitals after the diagnosis of CAC, so we had limited clinical data regarding therapy given for the entire period of treatment of IBD. However for most patients, we had a summary of their clinical course over the last few years before the diagnosis of CAC and a summary of major anti-IBD medications used (Supplementary Table 5). The majority of patients were described as having mild or quiescent IBD and were often not receiving continuous therapy for IBD. Five patients were described as having "severe disease". In five patients in the whole group, the diagnosis of CAC and IBD was made almost simultaneously. We categorized anti-IBD agents, which were used at any point in the treatment of IBD in these patients, as aminosalicylates, steroids, anti-TNF, and cytotoxics (*e.g.* 6-MP, methotrexate) (Supplementary Table 5). Of the 38 patients with known IBD prior to the time of cancer diagnosis for whom clinical data about IBD therapy was available, 74% had received aminosalicylates and 40% had used steroids. Use of cytotoxics (3 patients) and anti-TNF agents (4 patients) was uncommon.

Discussion

In this study, we find support for the hypothesis that there is a substantive genomic difference between CAC and sporadic CRC: genes commonly altered in CRC, such as *APC*, were much less commonly altered in CAC. Genes rarely altered in sporadic CRC, such as *IDH1*, were recurrently altered in CAC. The genomic differences we identified between CAC and sporadic CRC suggest that these tumors develop differently. As summarized by Ullman and Itzkowitz, the development of carcinoma in CAC appears to progress through a sequence consisting of indefinite dysplasia–low-grade dysplasia–high-grade dysplasia–carcinoma, in contrast to the classical progression of a discrete focus of neoplasia from a polypoid adenoma to an invasive carcinoma in sporadic CRC[21].

Novel findings in our NGS of a large series of CAC include recurrent *IDH1* R132 mutations, which we find only in the CD-associated cases, alterations of the FGFR pathway, and recurrent amplification of *MYC*. We note that the GA identified included those that may be targeted by agents already approved for other indications or in clinical trials. These include IDH1, ERBB2, EML4-ALK, and FGF pathway alterations. We also find that the spectrum of GA in UC CAC may differ from that of CD CAC: for example, besides *IDH1* mutations, *APC* alterations are significantly more common in CD-associated CAC than UC-associated cases. A limitation of our analysis is the use of targeted gene sequencing with NGS panels analyzing about 300 genes.

Recently, Robles *et al*[22] reported the results of an analysis, using whole exome sequencing, of 31 cases of CAC (in one patient, two distinct primary tumors were studied). Fifteen

patients had UC, 14 CD, and 2 indeterminate colitis. There were both similarities and differences between our study and the findings in Robles *et al* (shown also in Supplementary Table 6). As had Robles *et al*, we also find that the spectrum of GA in CAC is different from that found in sporadic CRC (in both studies, the TCGA-CRC dataset was used as a comparator for sporadic CRC). Both Robles *et al* and we note that there are also differences in recurrent GA between CAC arising in the setting of UC versus CAC arising in CD. There are also different findings in the two studies. Although in both studies *TP53* mutations were the most frequent alteration, the incidence of *TP53* alterations in Robles *et al* was similar to that of TCGA-CRC (63% versus 60%), whereas we found a much higher frequency of *TP53* mutations (89%). In agreement with Robles *et al*, we also find a lower frequency of *TP53* mutations in the hotspot residues 175 (1 mutation), 273 (1 mutation), and 245 (0 mutations). However, at variance with Robles *et al*, mutations in another hotspot, Arg248 (to either Trp or Gln), were the most abundant in our cohort, comprising 26% (8/31) of the total missense mutations found in these patients. The reasons for this difference between the two studies remain to be elucidated. We noted *MYC* amplification in 26% of patients higher than seen by Robles *et al*. (3%) or in TCGA-CRC (4%). Last, as discussed in more detail below, we found *IDH1* R132 mutations in 5/18 CD CAC patients. The differences in the incidence of GA seen between the two studies may be due to the relatively small number of patients studied; the higher coverage (500×) in the more focused NGS platform may have allowed us to identify less frequent variants.

As noted above, the *TP53* gene in our study was altered in 42 of 47 CAC cases, a much higher percentage than in sporadic CRC. Previous studies have identified *TP53* abnormalities as early events in the dysplasia-carcinoma sequence in CAC[9]. Our study supports this finding. In addition we note many alterations with potential GOF. The *TP53* alterations identified consisted largely of coding mutations, rather than truncating mutations, and included sixteen patients with neomorphic mutations that have been described as causing GOF activities, including enhancement of invasive properties, attenuation of apoptosis, and increased genomic instability[20]. It is possible that due to limited experimental data, some of the other missense mutations might also possess GOF capacity. This remains to be formally demonstrated. A recent study by Cooks *et al* suggests that the early presence of mutant p53 in the inflamed colon of IBD patients may actually be a driver of the subsequent progression to carcinoma by invigorating inflammation in the immediate microenvironment of the cells harboring mutant p53[7]. In this study, Cooks *et al* found that mice with mutant *TP53* exposed to dextran sodium sulfate, which elicits a condition resembling human IBD, not only developed more frequent inflammation-associated colon cancer, but also developed carcinoma much earlier than mice with knockout of one *TP53* allele, suggesting that mutant p53 may not only make the mice more susceptible to chronic inflammation, but also accelerate the development of carcinoma on an inflammatory background. Both of the *TP53* mutations (*TP53* R273H and *TP53* R175H) implicated in their study as actively contributing to the development of CAC were found in the tumors sequenced in our patients with CAC.

We identified *MYC* amplification in 26% of CAC cases, a substantially higher frequency than seen in sporadic CRC. The higher incidence of *MYC* amplification in our CAC cases may be due to the relative low frequency of activation of the canonical WNT pathway in

these cases. Mutational activation of the canonical WNT pathway is a very early event in sporadic colorectal carcinogenesis[11]. This is expected to lead to early transcriptional upregulation of Myc expression[23], greatly obviating the selective pressure for *MYC* gene amplification. Indeed, *MYC* gene amplification is relatively infrequent in sporadic colorectal cancer[17]. In contrast, in CAC, WNT pathway activation is less common; in those cases where it does occur, this is believed to be a late event[21]. This is likely to provide a selective drive for *MYC* gene amplification, providing a plausible explanation for the markedly higher frequency of such events in CAC. We note that in our study only 2/11 *MYC* amplified cases were coincident with *APC* mutations.

In the pathway analysis we performed, we found that several of the same pathways were altered in sporadic CRC and in CAC, but there were some significant differences. While p53 signaling was altered in 60% of CRC cases, p53 alteration appeared to be a near universal feature of the CAC. RTK/RAS signaling was activated in the majority of both sporadic CRC and CAC, with a similar frequency of RAS mutations, but a slightly higher frequency in CAC of activation of receptor tyrosine kinases, such as EGFR, ERBB2, and FGFR1/2; PI3K activation occurred in a large subset of both sporadic CRC and CAC. WNT/MYC signaling, which is altered in nearly all sporadic CRC, was altered in two-thirds of CD-associated CAC and one-third of UC-associated cases. We also note some differences in the pathways altered between CD and UC-associated CAC: In addition to the more frequent alterations in WNT/MYC signaling in CD-CAC, RTK and PI3K pathway alterations appeared more common in CD-CAC.

We found that targetable genomic alterations were frequent in CAC (36% of cases); agents targeting these alterations are already FDA-approved for other indications or are currently in clinical trials. Many of the potentially targetable alterations identified would not have been predicted based on previous genomic studies in sporadic CRC. For example, *IDH1* mutations, which are extremely rare in sporadic CRC, were significantly enriched in the CAC. This finding is consistent with a recent report from Hartman *et al* where they found *IDH1* mutations in 3/23 (13%) of CAC cases analyzed, 2 with CD and 1 with UC[24]. They used PCR-based sequencing to genotype *IDH1* at codon 132 and *IDH2* at codon 172 in CAC from 12 patients with CD and 11 with UC. In their more limited study, they also evaluated *KRAS* and *BRAF* mutation status in a subgroup of patients (a broader NGS platform was not used). Interestingly, recurrent *IDH1* mutations were first identified in glioblastomas, where they occur as an early event in the development of secondary glioblastomas that often harbor *TP53* mutations[25].

The presence of *IDH1* activating mutations only in the CD cases analyzed in our series suggests a distinct pathogenesis for CAC in CD compared to UC and also has potential therapeutic implications. *KRAS/BRAF* mutations were more common in the UC cases, raising the possibility that activation of this mitogenic pathway may play a role in the development of CAC in the setting of UC. As RAF and MEK inhibitors have recently been developed and are now entering the clinic, these findings if validated in larger studies may offer additional therapeutic targets.

In summary, our data support the hypothesis that CAC is a biologically different malignancy from sporadic CRC. A better understanding of the genomic events underlying the development of CAC could have implications for early detection of CAC in patients with IBD and for new therapeutic options for more advanced CAC. Different *TP53* mutations may confer a different risk profile for the development of frank carcinoma in dysplastic lesions. The identification of "second genomic hits" in dysplastic lesions, the majority of which already have *TP53* mutation, may also serve as biomarkers guiding management. For example, the identification of either an activating *TP53* mutation or a second genomic event in a dysplastic lesion may guide the extent of regional intervention, *e.g.* surgical resection instead of endoscopic mucosal resection. Further, our data suggest that there may be a spectrum of differences in genomic alterations between UC and CD cases and raises the possibility that *IDH1* mutation may be both a marker for CD-associated CAC and a promising therapeutic target.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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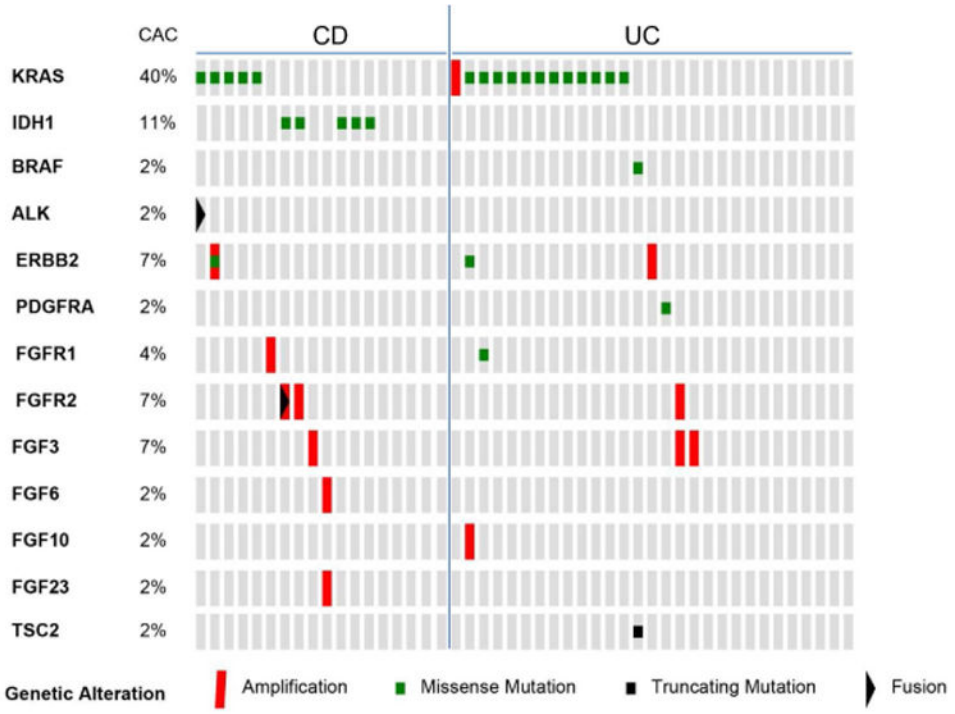

Figure 1.

Oncoprint showing genes mutated in at least 10% of CAC cases. Each column denotes an individual tumor and each row represents a gene. Colors indicate type of genomic alteration as indicated in the legend below the oncoprint.

Gene	CAC (n=47)	TCGA-CRC (n=212)	FM-CRC (n=3117)	CAC *v* TCGA-CRC	CAC *v* FM-CRC
	% altered			*p*-value	
TP53	89%	52%	75%	**<0.001**	**0.02**
APC	21%	76%	76%	**<0.001**	**<0.001**
KRAS	40%	42%	53%	0.7	0.07
SMAD4	17%	14%	16%	0.8	1
MYC	26%	4%	9%	**<0.001**	**<0.01**
GNAS	13%	11%	6%	0.6	0.06
IDH1	11%	1%	0.60%	**<0.01**	**<0.001**

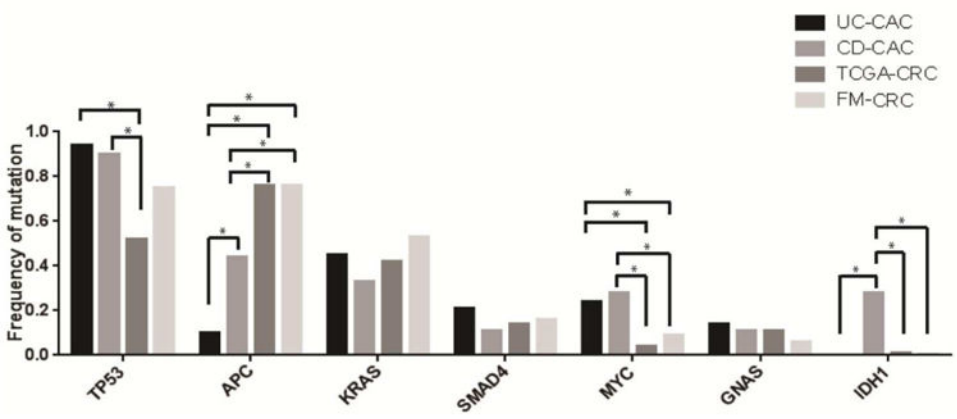

Figure 2.

Comparative analysis of the frequency of alterations in recurrently altered genes in Colitis-Associated Cancers (CAC) and in sporadic colorectal cancer. *Top:* Table showing the frequency of alterations in the indicated genes in the CAC cases overall (UC-associated plus CD-associated) versus the frequency of alterations in the same genes as found in the TCGA-CRC, and in the Foundation Medicine database (FM-CRC), and associated *p*-values based on Fisher's exact test. *Bottom:* Bar graph showing the relative frequency of genomic alterations in the indicated genes in CAC associated with UC (UC-CAC), CAC associated with CD (CD-CAC), TCGA-CRC, and FM-CRC. Differences in the frequency of alterations that were statistically significant, based on Fisher's exact test, are indicated with a star.

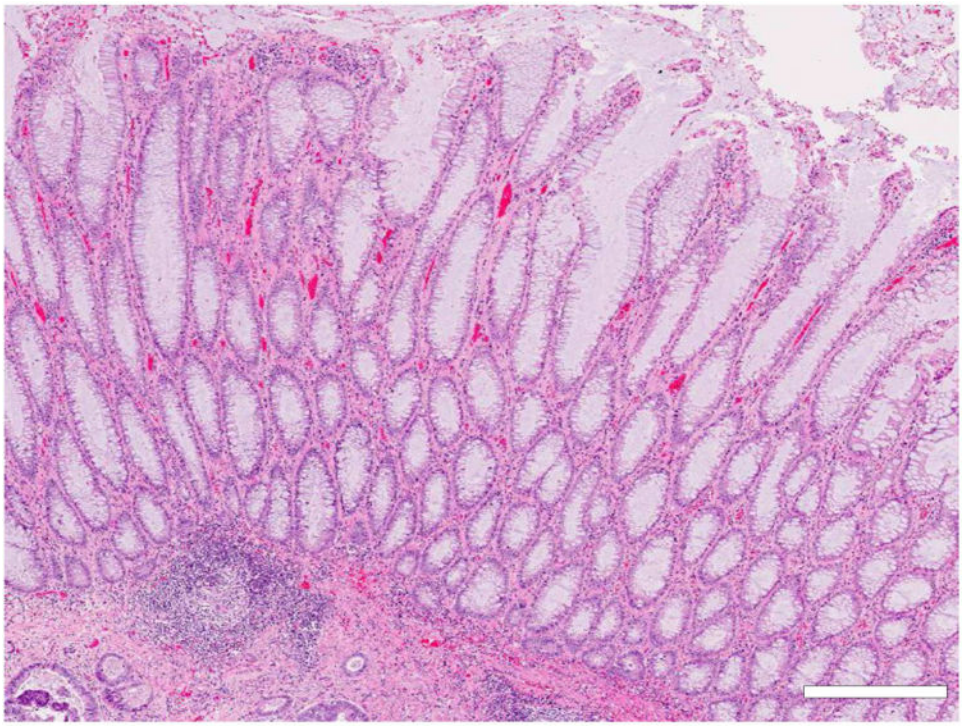

Figure 3.

Photomicrograph of rectal mucosa adjacent to tumor that shows no active colitis in a patient with history of quiescent CD and a tumor genomic alterations analysis mutation profile consistent with sporadic CRC. The horizontal sizing bar indicates 500um.

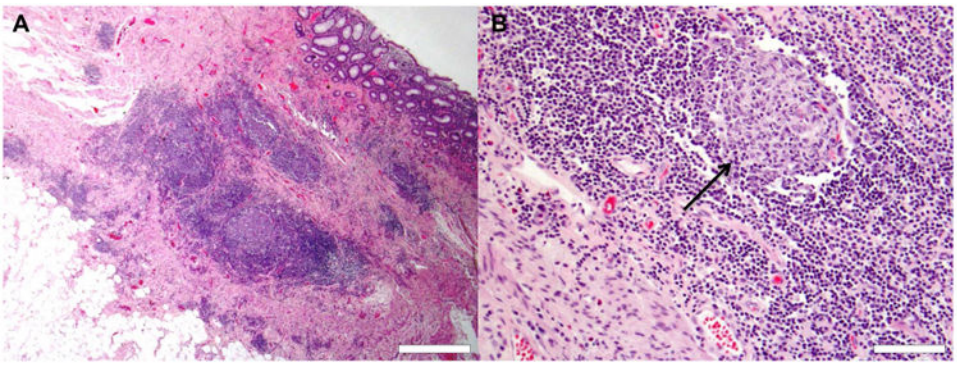

Figure 4.

Photomicrographs of colon mucosa showing (A) transmural chronic colitis and (B) granuloma formation (arrow), histologic changes characteristic of Crohn's colitis, in a patient with clinical history of UC, but found to have tumor *IDH1* R132 mutation. The horizontal sizing bars indicate 500um.

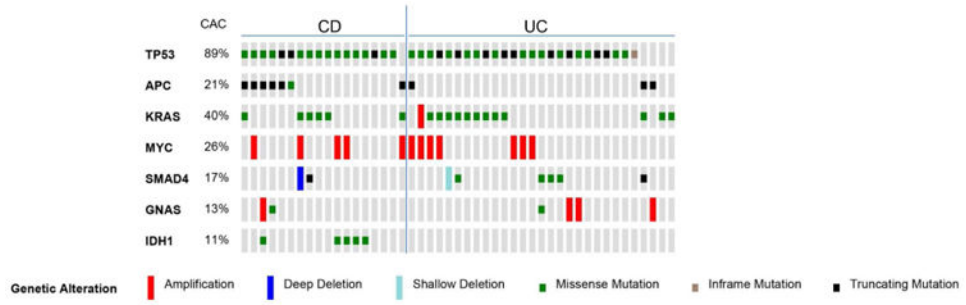

Figure 5.

Oncoprint showing alterations in genes that are potentially actionable, defined as genes whose altered product can be targeted either by agents that are already FDA-approved for other indications or are currently in clinical trials. Each column denotes an individual tumor and each row represents a gene. Colors and symbols indicate type of genomic alteration as indication in the legend below the oncoprint.

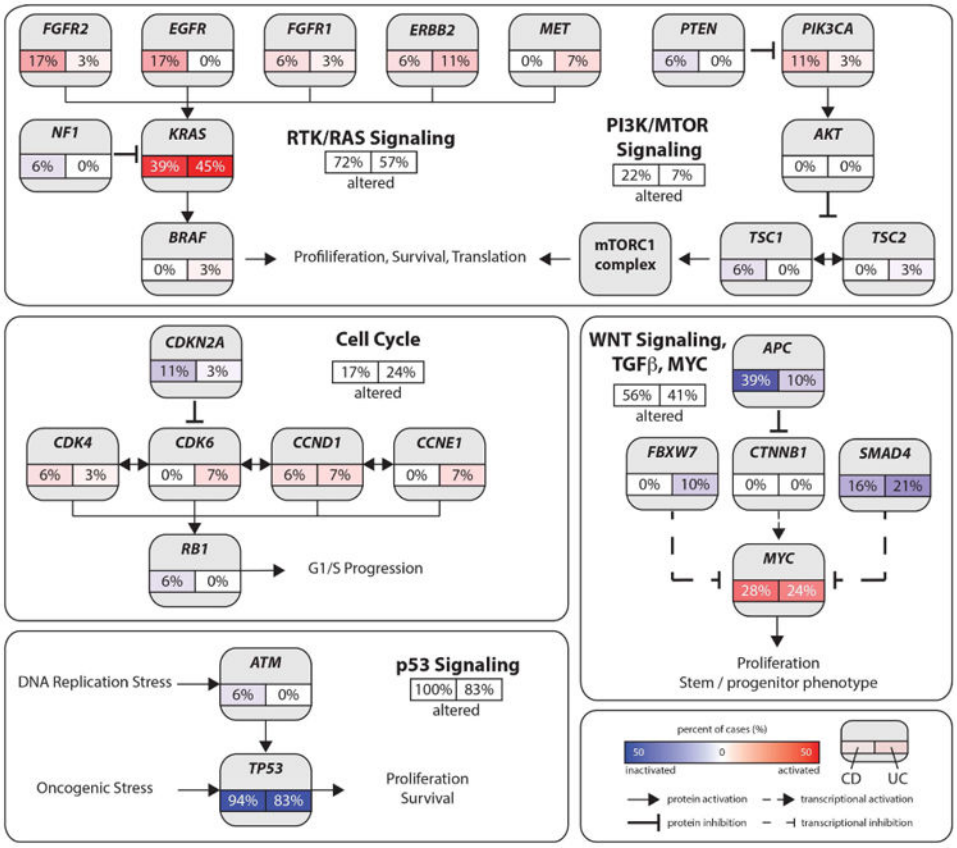

Figure 6.

Pathway alterations diagram integrating gene mutations and copy number alterations to identify pathways altered in UC- and CD-associated CAC. Alteration frequencies are expressed as a percentage of CD-associated cases (left side of box) and of UC-associated cases (right side of box). Red denotes activated genes and blue denotes inactivated genes, with the brightness of these colors corresponding to the percentage of cases altered. Amplifications and missense mutations in recurrent positions in oncogenes were considered activating, and deletions, truncating mutations as well as recurrent missense mutations in tumor suppressor genes were considered inactivating.

Table 1

Clinical characteristics

Characteristics	Colitis-Associated Bowel Cancers (n=47)	
IBD diagnosis	Ulcerative colitis	62% (n=29)
	Crohn's disease	38% (n=18)
Duration of IBD at time of CAC diagnosis	<10 years	21% (n=10)
	≥10 years	79% (n=37)
Patients' age at diagnosis	Mean	54 years (22-79)
	Median	55 years
Patients' gender	Male	51% (n=24)
	Female	49% (n=23)
Site of primary tumor	Ileum	4% (n=2)
	R colon	38% (n=18)
	L colon	13% (n=6)
	Rectosigmoid/rectum	45% (n=21)
Site analyzed for genomic alterations	Primary tumor	91% (n=43)
	Metastasis	9% (n=4)
Stage at diagnosis	Stage 0 (carcinoma in situ)	4% (n=2)
	Stage I	30% (n=14)
	Stage II	23% (n=11)
	Stage III	28% (n=13)
	Stage IV	15% (n=7)