TOPIC HIGHLIGHT

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Colitis-associated colon cancer: Is it in your genes?

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Abstract

Colitis-associated colorectal cancer (CA-CRC) is the cause of death in 10%-15% of inflammatory bowel disease (IBD) patients. CA-CRC results from the accumulation of mutations in intestinal epithelial cells and progresses through a well-characterized inflammation to dysplasia to carcinoma sequence. Quantitative estimates of overall CA-CRC risks are highly variable ranging from 2% to 40% depending on IBD severity, duration and location, with IBD duration being the most significant risk factor associated with CA-CRC development. Recently, studies have identified IBD patients with similar patterns of colonic inflammation, but that differ with respect to CA-CRC development, suggesting a role for additional non-inflammatory risk factors in CA-CRC development. One suggestion is that select IBD patients carry polymorphisms in various low penetrance disease susceptibility genes, which predispose them to CA-CRC development, although these loci have proven difficult to identify in human genome-wide association studies. Mouse models of CA-CRC have provided a viable alternative for the discovery, validation and study of individual genes in CA-CRC pathology. In this review, we summarize the current CA-CRC literature with a strong focus on genetic predisposition and highlight an emerging role for mouse models in the search for CA-CRC risk alleles.

Key words: Colitis-associated colorectal cancer; Inflammatory bowel disease; Forward genetics; Susceptibility genes; Azoxymethane; Dextran sulfate sodium; Mouse models

Core tip: Colitis-associated colorectal cancer (CA-CRC)

is the cause of death in 10%-15% of inflammatory bowel disease (IBD) patients. Quantitative estimates of overall CA-CRC risk are highly variable and depend of the severity, duration and location of active IBD. Recently, studies have identified IBD patients with similar patterns of colonic inflammation, but that differ with respect to CA-CRC development, suggesting a role for additional non-inflammatory risk factors in CA-CRC development. In this review, we summarize the current CA-CRC literature with a strong focus on genetic pre-disposition and highlight an emerging role for mouse models in the search for CA-CRC risk alleles.

INTRODUCTION

Inflammatory bowel disease (IBD) is an umbrella term used to describe chronic-relapsing inflammatory conditions of the intestinal tract[1]. While there are several subtypes of IBD, the two most common are Crohn's disease (CD) and ulcerative colitis (UC). CD is characterized by inflammation throughout the entire gastro-intestinal tract with lesions most commonly found in the small intestine and proximal colon. Approximately 60% of CD patients have colonic involvement, with only 20% having isolated colonic disease[2]. In CD, the inflammation is transmural, traversing multiple layers of the intestine, and typically occurs in patches[1]. In UC, inflammation arises in the rectum and spreads proximally in a continuous manner, rarely extending into the small intestine and is confined to the mucosal layer. Worldwide IBD incidence rates are highly variable (UC: < 1-24.3/100000, CD: 1-20.2/100000), with higher incidence recorded in Western and Northern Europe, Australia and North America and lower incidence in Africa (excluding South Africa), Asia and South America[3-5]. A large-scale meta-analysis of 107 IBD studies (57 CD and 50 UC) recently determined that CD incidence increased in 75% of CD studies and 60% of UC studies over a period of at least 10 years[4]. As IBD patients exhibit a low mortality rate, the global prevalence of IBD is expected to increase in the coming years.

Colitis-associated colorectal cancer (CA-CRC), which develops in areas of active colonic inflammation, is listed as cause of death in 10%-15% of all IBD patients[6,7]. As rates of IBD increase, rates of subsequent CA-CRC are also predicted to increase. From a colon cancer perspective, inflammation is the third most common CRC risk factor, after the hereditary CRC syndromes familial adenomatous polyposis coli (FAP) and hereditary non-polyposis colon cancer (HNPCC). However, unlike FAP and HNPCC, whose etiologies are well characterized, the specific etiologies underlying increased CA-CRC are still being elucidated. In this review, we briefly highlight the current literature with respect to CA-CRC etiology and epidemiology and compare and contrast CA-CRCs relative to non-inflammatory CRC conditions and IBD. In addition, we speculate on a possible function for genetic pre-disposing risk factors in CA-CRC and a role for animal models in elucidating these genetic effects.

EPIDEMIOLOGY, ETIOLOGY AND SURVEILLANCE

CA-CRC is listed as cause of death in 10%-15% of IBD patients[7]. CA-CRC mortality is approximately 50% (CD: 46%, UC: 50%) and this suggests that between 20%-30% of IBD patients will develop CA-CRC within their lifetime[6]. Both UC- and CD-CRC are early-onset conditions presenting with an average age of onset between 40-55 years of age[6,8-10]. UC-CRC is primarily identified in the rectum and sigmoid colon, whereas CD-CRC is more evenly distributed between the right-colon (ascending), sigmoid colon and rectum, although only a small proportion of CD patients have colonic disease[6,11]. The differences with respect to tumor location may reflect differences in location of active IBD as 76% of CD-CRCs and 100% of UC-CRCs arise in areas of macroscopic IBD. CA-CRC patients often present at diagnosis with multiple synchronous carcinomas (CD-CRC: 11%, UC-CRC: 12%) and with lesions showing a high proportion of mucinous and signet ring features (CD-CRC: 29%, UC-CRC: 21%)[6].

According to the American Cancer Society, individuals at increased risk for CA-CRC should undergo routine colonoscopy at 1-2 year intervals starting 8-12 years post-disease diagnosis (www.ccfa.com). It is also recommended that at least four random colonic biopsies be taken for every 10 cm of colon examined during these routine colonoscopies, as approximately 20%-50% of colon dysplasia cannot be detected by visual inspection alone[12,13]. Intraepithelial neoplasms are highly variable with respect to appearance and may present as raised (pedunculated or sessile) or flat (plaque or bump) lesions[14]. Flat lesions are a unique feature to CA-CRC, rarely being detected in familial or sporadic CRC, and are generally associated with high risk of transformation into CA-CRC[15]. The identification of CA-CRC can also be further complicated by large benign inflammatory pseudopolyps, which form during mucosal regeneration and ulcer healing.

Inflammation in CA-CRC pathogenesis

Quantitative estimates of overall CA-CRC risks are highly variable ranging from 2% to 40% depending on IBD severity, duration and location[7]. CD patients with disease isolated to the small intestine only are not at increased risk of CD-CRC supporting the strong

link between inflammation and CA-CRC[6]. CRC risk in UC has been estimated at 2% after 10 years, 8% after 20 years and 18% after 30 years of disease[8]. Studies of UC-CRC have also noted a high concordance between CA-CRC risks with location/extent of disease. For example, Ekbom et al[16] identified a standardized incidence ratio (SIR) of 1.7 for proctitis (rectal only), 2.8 for left-sided colitis and 14.8 for pancolitis (defined as extensive colitis, or colitis involving the entire colon).

Studies of CD-CRC are complicated by vast heterogeneity with respect to CD anatomical sites. However, as with UC, CA-CRC risk associations have been correlated with duration/severity of disease. The relative risk (RR) of CD-CRC based on duration of disease was calculated to be 2.9, 5.6 and 8.3 after 10, 20 and 30 years of disease, respectively[17]. In 2007, a CRC meta-analysis by disease site estimated a RR of 0.85, 4.3 and 13.4 for small bowel only, ileocolic and colon CD, respectively[18]. CD-CRC RR is increased to 18.2 in patients with extensive disease.

One of the oldest and most prevalent treatments in IBD is administration of the non-steroidal anti-inflammatory (NSAID) drug 5-aminosalicylic acid (5-ASA) or its derivatives. 5-ASA modulates mucosal inflammation through several mechanisms including: the down regulation cyclooxygenase 2; inhibition of tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β); decreased nuclear factor kappa beta (NF-$\kappa\beta$) activation and modulation of peroxisome-proliferator activated receptor gamma (PPAR-γ)[19]. While the protective effects of 5-ASA in IBD are well established, the literature examining 5-ASA as a preventative agent in CA-CRC is controversial. Some studies have demonstrated up to a 97% reduction in CA-CRC risk in patients receiving regular 5-ASA therapy[20-22]. However, recent studies tend to support no protective effects of regular 5-ASA use on CA-CRC risk[23-25]. These discrepancies highlight the complex nature of CA-CRC. It also leads to questions regarding whether there may be certain non-inflammatory factors, such as genetic predisposition that may influence the efficacy of 5-ASA therapeutics.

CA-CRC initiation and progression is dependent on the accumulation of mutations in various tumor suppressors and oncogenes in intestinal epithelial cells[26]. Support for inflammation as a key mediator in CA-CRC pathogenesis comes from animal studies showing increased DNA damage and tumor formation following extended periods of colitis in mice in the absence of a known DNA mutagen[27]. The specific mechanisms through which inflammation regulates CA-CRC initiation and progression are not well understood. It has been suggested that reactive oxygen species (ROS) produced by immune cells during colitis may play a crucial role in promoting DNA damage. Epigenetics, cytokines and the microflora are also thought to be important in mediating cross talk between increased inflammation and CA-CRC and are reviewed in[28].

Primary sclerosing cholangitis
Primary sclerosing cholangitis (PSC) is a rare disease characterized by inflammation, fibrosis and subsequent narrowing of the common bile duct. This narrowing leads to the accumulation of bile in the liver resulting in cirrhosis and future liver failure thus reducing life expectancy[29]. There is a strong correlation between IBD and PSC, with approximately 70% (CI: 46.5% to 98.7%) of PSC patients presenting with concomitant IBD, usually in the form of UC[30]. This corresponds to 8% of IBD patients developing coexisting PSC[31,32]. The specific etiology underlying PSC development is complex, but similar to IBD is thought to arise due to a combination of genetic, environmental and microbial risk factors[33,34].

In 2002, a large-scale meta-analysis concluded that PSC patients were at increased risk of developing CA-CRC compared to both IBD patients without PSC and the general population[35]. While there has since been conflicting data concerning CA-CRC in PSC-IBD patients[32], it is generally accepted that PSC is a risk factor associated with CA-CRC development. The explanation behind increased CRC in PSC-IBD patients remains elusive, but may be associated with increased levels of bile acid. Co-diagnosis of IBD and PSC is important to clinicians, as there is some evidence to suggest that treatment with ursodeoxycholic acid (UDCA) may reduce risk of CA-CRC, although additional testing is still necessary[36].

Evidence for non-inflammatory factors in CA-CRC pathogenesis
In addition to strong evidence linking extent and duration of colonic inflammation to CA-CRC risk in IBD patients, there have recently been several observations in humans and mice to suggest a role for non-inflammatory factors in CA-CRC initiation/progression. Family history of CRC development is an important parameter to assess in IBD patients as a positive family history of CRC is associated with a 2-fold greater risk of developing CA-CRC[37]. Studies of human UC and CD-CRC have also shown increased risk of CA-CRC in patients diagnosed with IBD at a young age. For UC-CRC, the absolute CRC risk 35 years post-diagnosis was 40% vs 30% in early-onset (age 15 or less) and late-onset UC patients diagnosed with pancolitis, respectively[16]. This was subsequently confirmed in a large scale meta-analysis whereby patients with UC diagnosed prior to 25 years of age were 13 and 70 times more likely to develop CA-CRC compared to older UC patients and the general population, respectively[38]. A similar trend was seen in CD with an increased RR of 21.5 vs 1.6 in patients younger and older than 25, respectively and subsequently confirmed in second unrelated CD cohort[18,39]. The specific etiology underlying increased CA-CRC risk in younger onset IBD is still being investigated.

In 2014, Connolly et al[40] compiled a cohort of

UC patients to study the role of select IBD genes in CA-CRC. In this study, two cohorts were identified; patients with CA-CRC and those without, despite having similar amounts of UC-inflammation in both cohorts. Similar observations in mice with high levels of colonic inflammation, but low levels of CA-CRC have also been reported and will be discussed further in the mouse model section of this review[41,42]. CA-CRC divergence among individuals with similar IBD status suggests a role for other non-inflammatory factors in mediating CA-CRC initiation or progression and it has been suggested that similar to many other complex traits, select IBD patients are genetically pre-disposed to developing CA-CRC.

GENETIC ASSOCIATIONS IN HUMAN CA-CRC

The "common disease, common variant" hypothesis, stipulates that common complex diseases, such as cancer, diabetes and IBD, arise in part due to common genetic variants (single nucleotide polymorphisms, SNPs) within the genome[43]. To understand the rationale for hypothesizing genetic predisposition in CA-CRC, we must reiterate the similarities with respect to cancer progression between CA-CRC and a type of non-inflammatory CRC, often referred to as familial CRC.

Familial vs colitis-associated colorectal cancers

Genetically, CRCs can be categorized on a sliding scale of pre-disposing risk, which describes the predicted effect size of a given CRC risk variant compared to the minor allele frequency [(MAF), the abundance of the minor allele within a reference population][44]. At one extreme, there are the rare, but well-characterized Mendelian or Hereditary CRC syndromes, such as FAP or HNPCC, whose mutations are associated with a high penetrance of disease symptoms and are easily identified in large families with multiple affected individuals. At the other extreme are familial CRCs, which present with fewer affected individuals per family, and arise, in part, due to common genetic variants within a class of genes known as low penetrance tumor susceptibility genes[45].

In familial CRCs, like most cancers, the balance between cell proliferation, differentiation and apoptosis becomes progressively disrupted through the accumulation of mutations in several signaling pathways encompassing *WNT*, *RAS*, *p53*, *DCC* and *TGF-β* genes. This is referred to as the adenoma-carcinoma sequence progression[46]. Analysis of invasive familial and CA tumors show a similar pattern of acquired molecular alterations and hence CA-CRC was originally categorized as a subtype of familial CRC. This led to speculation that low penetrance tumor susceptibility genes, which are important in familial CRC, could also be important in CA-CRC initiation and progression.

However, the timing and frequency of these genetic events differ between familial and CA-CRC and therefore it has been hypothesized that variants in different genes may be associated with both cancers. For example, mutations/deletions of *p53* are early events in CA-CRC with 50% of ulcerative colitis (UC) patients having *p53* mutations compared to approximately 10% of non-inflammatory adenomas (Figure 1)[28,47]. But *APC* mutations are rare events in CA-CRC (27.5% of high grade dysplasia) compared to 50% in non-CA-CRC adenomas[28,48].
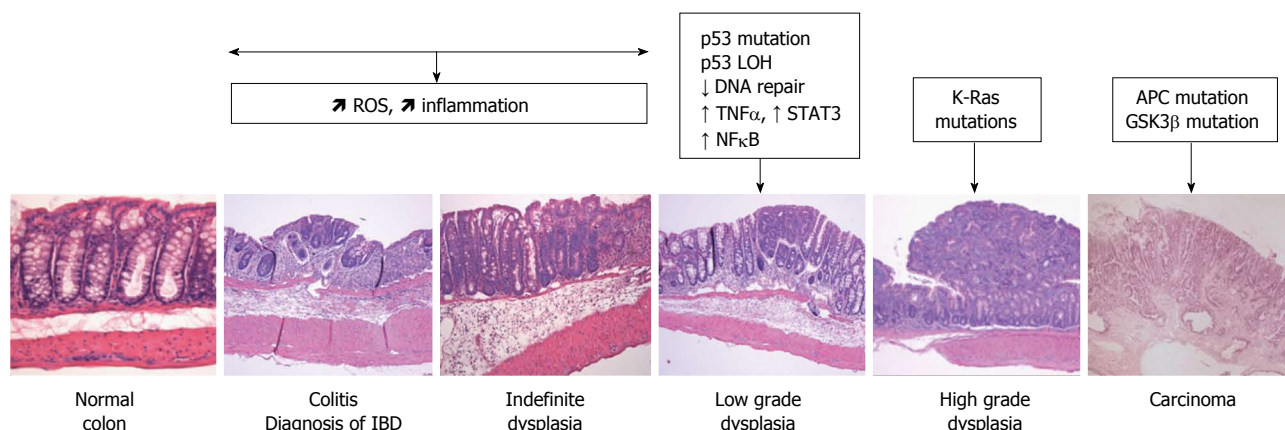
CA-CRCs progress through the colitis-dysplasia-carcinoma sequence associated with the development of inflammation, indefinite, low-grade and high-grade dysplasia, with eventual progression to carcinoma (Figure 1)[26]. Dysplasia describes the abnormal growth and development of colon cells, with indefinite dysplasia describing early changes that cannot be categorized as either negative or positive for dysplasia. It is interesting to note that key inflammatory mediators such as reactive oxygen and nitrogen species (ROS and NOS), as well as chemokines and cytokines (IL-6, STAT3, TNF-α, IL-10, IL-12 and IL-23) all participate to orchestrate the conversion of a normal epithelium to indefinite dysplasia, which again highlights a variable role for inflammation in CA-CRC transformation[28].

Genome-wide association studies

The completion of the Human Genome Project, the International HapMap Project and increased technological power has led to the advent of genome-wide association studies (GWAS)[49]. GWAS compare the prevalence of thousands of common genetic variants [single nucleotide polymorphisms (SNPs)] within healthy (control) and disease (case) cohorts looking for allelic imbalance indicative of disease association[49].

Both IBD and familial CRC have been associated with polymorphisms in low penetrance disease susceptibility genes, with numerous positive associations detected in GWAS. For IBD, more than 200 loci have been identified, the largest number for any common complex disease (http://genome.gov/gwastudies). As IBD pathogenesis is driven by aberrant immune responses against the commensal bacteria of the lumen, it is not surprising that a large number of genes within IBD loci have been associated with epithelial barrier maintenance and permeability, cytokine signaling and pathogen recognition/clearance[50]. Some of the most well characterized genetic associations are *NOD2*, *IL-23R* and *ATG16L1* involved in bacterial sensing, the IL-23 inflammatory response and autophagy, respectively[51]. To date more than 40 loci have been associated with familial CRC (http://genome.gov/gwastudies), with many of the SNPs mapping to regions in strong linkage disequilibrium (LD) with members of the TGF-β signaling pathway,

Figure 1 Progression of colitis-associated colorectal cancer. Colitis-associated colorectal cancer progresses through a colitis-dysplasia-carcinoma sequence associated with the development of inflammation, low-grade, high-grade dysplasia and eventually carcinoma due to molecular alterations. IBD: Inflammatory bowel disease; ROS: Reactive oxygen species.

highlighting an important role for TGF-β signaling in CRC[52]. There is little overlap between known IBD and CRC loci in humans suggesting different etiologies to both diseases.

Lack of GWAS for colitis-associated colon cancer

Unlike familial CRC and IBD, there have been very few GWAS performed to identify genetic loci regulating susceptibility to CA-CRC. In part, this may be due to high numbers of IBD patients undergoing colectomy, making identification of IBD patients with and without CA-CRC difficult[53,54]. CA-CRC is influenced by numerous risk factors including age at IBD-onset, and duration/extent of IBD colonic involvement[7,38]. While not essential for early CA-CRC GWAS it may also be important to segregate CA-CRC patients into categories associated with differences with respect to age of diagnosis, ethnicity and extent of inflammation, as different genes may underlie CA-CRC in different colonic microenvironments.

In 2009, the UK IBD Genetics Consortium identified and published a novel UC locus situated on chr. 16 (16q22)[55]. Interestingly, this locus had previously been associated with increased CRC risk[56]. Therefore, it has been speculated that this locus may also play an important role in CA-CRC. However, a recent study showed no association between any known UC loci and UC-CRC risk, disproving this hypothesis[40]. Recently, the STAT3 locus has been associated with both IBD and CA-CRC, exerting its effects in a TP53-dependent manner. This is a promising step in the identification of CA-CRC loci in humans[57].

MOUSE MODELS OF COLITIS-ASSOCIATED COLON CANCER

The complex and heterogeneous genetic component of complex diseases can be difficult to tease apart in human populations due to confounding environmental and lifestyle variables. However, these traits can

be dissected in genetically well-defined inbred and recombinant congenic mouse strains[58]. Mice are not particularly prone to the spontaneous development of IBD or CRC and therefore disease induction in mice can be performed using dietary modifications, infectious agents, genetic mutation or chemical reagents[59]. To date more than 100 different mouse models of CRC, IBD, and CA-CRC have been published. For a comprehensive review of these, see[58,60-62].

We have narrowed the focus of this review to three relevant areas; the *Il-10* knockout genetic model of colitis/CA-CRC, the AOM/DSS model of CA-CRC and the mapping of genetic loci regulating susceptibility to CA-CRC using forward genetic approaches.

The Il-10 model of colitis and CA-CRC

Many common colitis and CA-CRC models involve deleting the expression of a specific gene or multiple genes. These models are associated with an increase in IBD (either UC or CD) with or without subsequent CA-CRC[60]. The most-well characterized of these genetic models involves the deletion of the *Il-10* gene encoding a pleiotropic anti-inflammatory cytokine produced by monocytes and lymphocytes that acts to dampen and terminate immune responses[63]. In 1993, Kuhn *et al*[64] generated the 129/B6 *Il-10$^{-/-}$* knockout mouse line (*Il-10tm1Cgn*). These mice showed a high incidence of weight loss, anemia and enterocolitis 1-3 mo after birth. Enterocolitis was first detected in the proximal colon and then in the remaining colon, the duodenum and the proximal jejunum of the small intestine and mimics human CD, associated with discontinuous, transmural inflammation, ulceration and thickening of the bowel wall[64-66].

Enterocolitis in *Il-10$^{-/-}$* mice is strain-dependent, suggesting a strong role for genetic factors in disease pathogenesis. The most sensitive genetic backgrounds are C3H/HeJBir and 129/Sv, with 100% of the mice developing severe colitis before 3 mo of age[66,67]. C3H/HeJBir mice, with a wild type *Il-10* gene are also

susceptible to spontaneous colitis[60]. However, CA-CRC susceptibility has not been assessed in the C3H/HeJBir *Il-10*^{-/-} mice[67]. On the 129/Sv background, 67% of the mice develop adenocarcinomas in the first 6 mo of life[66]. As evaluated by histopathology, BALB/cJ *Il-10*^{-/-} mice have a higher incidence of spontaneous colitis (100%) compared to B6 *Il-10*^{-/-} mice (57%) at 3 mo of age, but a lower incidence of colonic tumors (29%) at 6 mo of age compared to 129/Sv *Il-10*^{-/-} mice. B6 *Il-10*^{-/-} mice do not develop colonic adenocarcinomas within this timeframe. NOD/LtJ *Il-10*^{-/-} mice also develop severe colitis, associated with 100% incidence of rectal prolapse, although the time frame for disease development was not specified[68]. These NOD/LtJ *Il-10*^{-/-} mice are not good models for CA-CRC as high incidence of rectal prolapse prevents long-term studies in these mice. Together, these studies highlight an important role for genetic backgrounds in colitis and CA-CRC susceptibility.

Generally, experiments of colitis and CA-CRC in *Il-10*-deficient mice support a strong role for inflammation as the driving factor underlying increased CA-CRC risk. However, an exception to this is a study from Arthur *et al*[69] who demonstrated similar inflammatory profiles in *Il-10*^{-/-} mice infected with *E. faecalis* and *E. coli*, with only the latter being associated with increased CA-CRC, supporting a role for non-inflammatory mediators of CA-CRC.

The AOM/DSS model of CA-CRC

Chemical models of colitis and CA-CRC are advantageous as treatments are relatively inexpensive and easy to administer producing highly reproducible results. These models offer a distinct advantage compared to genetic models as time of onset, duration and severity of colitis/CA-CRC can be adjusted by changing the dose and/or length of the treatment protocol. In addition, unlike genetic models of colitis and CA-CRC, the inflammatory agents can be removed and thus the healing/regeneration process can be studied in detail. In addition, these models highlight a probable role for genetic factors in CA-CRC, with some mice developing high levels of colonic inflammation, yet low levels of CA-CRC and *vice versa*.

In 2003, Tanaka *et al*[70] published results showing that a single azoxymethane (AOM) injection in CD-1 mice, followed a week later by a 7-d dextran sulfate sodium (DSS) treatment, was sufficient to induce macroscopically visible tumors 20 wk post-initiation. Mice treated with only a single AOM or single DSS injection did not develop tumors within this 20-wk period, suggesting that combined administration of AOM and DSS is essential for tumorigenesis. This AOM/DSS protocol has since become one of the most popular models to study the influence of dietary, microbial and genetic factors of CA-CRC progression and initiation[58]. Interestingly, permissive mice given multiple injections of AOM develop CRC, reminiscent

of human familial CRC, while those given DSS-alone develop an UC-like phenotype. This allows for common and unique genetic signatures to be identified between the AOM/DSS CA-CRC protocol and the AOM-only CRC and DSS-only IBD protocols.

AOM is a colon specific carcinogen that, when activated, generates a methyl cation that can react with deoxyguanosine at either the N^7 or O^6 position; with the latter leading to the formation of deoxymethylguanosine, resulting in mismatched base pairing and subsequent G to A transitions. DSS is a long chain (5-140 kDa), negatively charged polysaccharide derived from the esterification of dextran and chlorosulfonic acid[71]. When administered to rodents in drinking water, DSS is a highly potent inducer of colitis, mimicking human UC[72]. The location of colitis is highly dependent on the DSS molecular weight, with low weight DSS (5 kDa) inducing lesions in the cecum and proximal colon, mid weight (40 kDa) DSS provoking lesions in the mid and distal colon and high weight DSS (500 kDa) failing to induce colitis in mice[73]. All future mention of DSS refers to mid-weight (approximately 36-54 kDa) DSS.

Inbred strains of mice differ with respect to AOM/DSS-induced CA-CRC susceptibility, with strains such as BALB/c, Swiss Webster, CBA/J, CD1, A/J and FVB/NJ behaving as susceptible and strains such as C3H/HeJ, C57Bl/6 (B6) and DBA/2J being resistant[70,74-78]. Testing for DSS-induced colitis in some strains, such as BALB/c, CBA/J and DBA/2J, suggests that the extent of colonic inflammation is an important driver for CA-CRC[41,72]. However, C3H/HeJ mice are highly susceptible to DSS-induced colitis, yet resistant to CA-CRC, suggesting that inflammation alone does not determine CA-CRC susceptibility[41]. We have also shown that A/J mice, while more susceptible to CA-CRC than B6 mice, develop lower levels of overall colonic inflammation compared to B6 mice following AOM/DSS treatment[78]. Studies of myeloid translocation gene, related 1 (*Mtgr1*) gene deficiency in mice have demonstrated reduced tumor burden following AOM/DSS treatment despite an increased colonic inflammation, again suggesting a role for non-inflammatory, possibly genetic factors in CA-CRC[79].

Recently, Gao *et al*[80] compared global gene expression patterns in untreated BALB/c inbred mice, to those treated with AOM/DSS, AOM-only and DSS-only. As expected, both the DSS- and AOM/DSS-treated mice showed evidence of increased colonic inflammation, which was notably absent in the AOM-only and untreated mice. However, despite the strong influence of inflammation in the AOM/DSS-treated mice, approximately 50% of the identified differentially expressed genes were unique to the AOM/DSS treatment group and were not observed in the AOM or DSS-only groups. Li *et al*[81] also recorded unique genetic signatures association with AOM/DSS-induced CA-CRC, compared to chronic murine colitis,

confirming this observation. Collectively, these studies suggest that CA-CRC susceptibility is associated with unique genetic signatures. Identification of genes specific to CA-CRC may aid in the identification of IBD patients with rapid onset CA-CRC or those who develop CA-CRC despite low levels of colonic inflammation.

Mouse loci identified using forward genetic studies

The identification of genes associated with the development of various complex traits, can be identified using either forward or reverse genetic approaches in mice. Forward genetics is a phenotype-driven approach in which mutations are identified underlying disease traits through the generation of informative mouse crosses followed by linkage analysis[82]. This is the converse of reverse genetics in which a range of phenotypes are characterized for a given genetic mutation[83]. Reverse studies are easier to conduct and are shorter in duration than forward genetic studies, but can be hampered by inefficient knockdown or genetic background effects[83,84]. In addition, forward genetic screens are advantageous as they are conducted without bias as to the types of mutations detected, with mutations mapping to genes that are often unlikely to be tested using reverse genetic approaches and represent a spectrum of mutations more likely to be detected in human disease. Forward genetic studies typically use 4 distinct types of mouse populations; F2, N2, recombinant congenic mice (RCS) or recombinant inbred mice (RI), often using more than one informative population.

Numerous non-inflammatory CRC and IBD susceptibility loci have been mapped in mice using a forward genetics approach and therefore, it is not improbable to hypothesize that CA-CRC loci could be identified using the same approach. These forward genetic approaches are possible as inbred mice differ with respect to susceptibility to all three of the above diseases. Figure 2 summarizes the known IBD, CRC and CA-CRC loci mapped using a forward genetics approach. With respect to IBD, these loci have been mapped using spontaneous (SAMP1/YitFC), chemical (DSS, TNBS), genetic (Il-$10^{-/-}$, $Gnai^{-/-}$, $Gpx1/2^{-/-}$) and infectious (*Helicobacter, Trichuris muris*) models of colitis, while non-inflammatory CRC loci have been mapped using the $Apc^{Min+/-}$ mouse model of CRC (mimicking FAP) and AOM (or the AOM precursor dimethylhydrazine)-only models[42,85-101]. Despite differences with respect to strains of mice tested and models of disease induction used, these studies share a common feature, *i.e.*, each cross identified multiple genetic loci regulating susceptibility and each locus controls a small proportion of the phenotypic variation (< 20%).
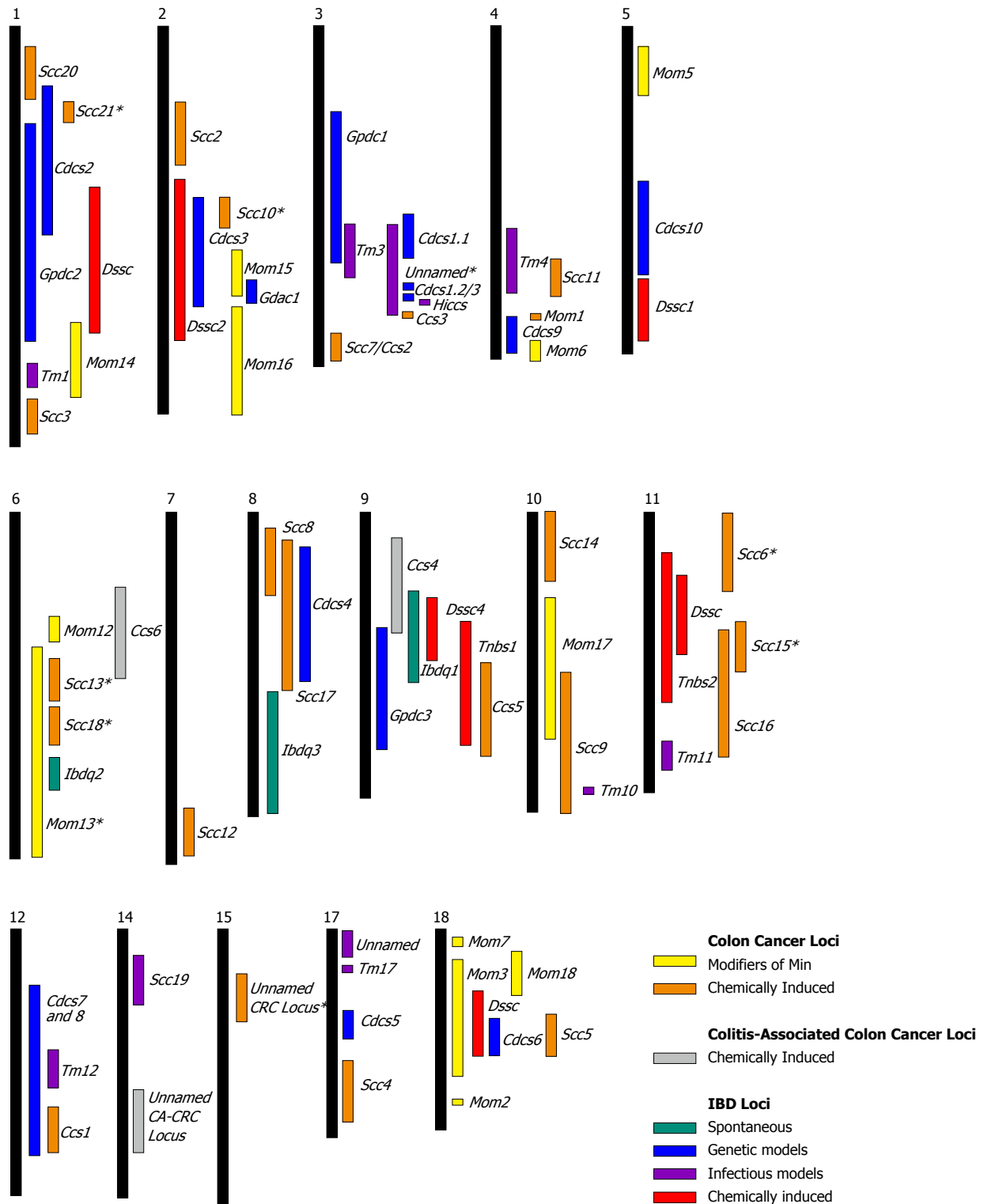
However, only 3 CA-CRC loci have been identified (Figure 2). The first locus referred to as *Hiccs*, regulates *Helicobacter hepaticus*-induced colitis and CA-CRC susceptibility[88]. This *Helicobacter* model however, is a poor recapitulation of human disease, with mice developing lesions exclusively in the proximal colon.

Our laboratory has also mapped two additional loci that regulate CA-CRC susceptibility. To map these loci, we first defined that A/J mice, contrary to B6 mice, were susceptible to AOM/DSS-induced CA-CRC. Then, using forward genetics and (A/J X B6) F1 and F2 cohorts, we identified and mapped a novel A/J-derived CA-CRC susceptibility locus to mouse chromosome 9, centered around marker D9Mit67. This novel locus was named *Ccs4*[78]. Further analyses of (A/J X B6) F2 mice homozygous for A/J alleles at *Ccs4* identified a second locus on the distal part of mouse chromosome 14 (peak marker rs13482311, 93.5 Mb) that acts to regulate tumor susceptibility in an additive fashion with the *Ccs4* locus. F2 mice homozygous for A/J alleles at both loci (*Ccs4* and chromosome 14) are as susceptible to CA-CRC as the A/J controls, while mice homozygous for B6 alleles are as resistant as the B6 controls, thus supporting the role of two loci in this CA-CRC model. Two locus systems are rarely identified in human GWAS studies, in part due to the low penetrance of the second locus. The ability to detect such interactions in mice provides a framework to search for such associations in humans. In our studies, we also detected higher levels of inflammation in the resistant B6 colons, suggesting that elevated inflammation is not the primary driver of this differential CA-CRC susceptibility. It is interesting to note that an unrelated locus on chromosome 3, namely *Ccs3*, is the primary driver of AOM-induced CRC susceptibility in these same strains, suggesting that these CRC loci may be specific to CA-CRC[102]. The success of this initial genetic screen has led us to hypothesize that other novel genetic factors may also regulate susceptibility to CA-CRC in different inbred mouse strains, which we are currently assessing.

CONCLUSION

CA-CRC is a complex disease arising from a combination of dietary, lifestyle, microbial and genetic factors. In addition, disease risk is tightly correlated with severity, location and duration of colonic inflammation (IBD). CA-CRC risk is increased in early-onset IBD patients and this specific subset of IBD patients is increasing in North America, suggesting that CA-CRC may be a growing concern for future generations[103]. It has been well established that various reverse genetic approaches are ideal for identifying genes associated with increased inflammation and subsequent CA-CRC. However, we have recently shown that we can use forward genetics and the common AOM/DSS model of CA-CRC to identify and map novel loci regulating susceptibility to CA-CRC. By identifying parental strains for mapping, discordant with respect to their colitis and CA-CRC phenotype,

Figure 2 Mouse inflammatory bowel disease and colorectal cancer susceptibility loci. Summary of the current inflammatory bowel disease and colorectal cancer (CRC) loci mapped in inbred mice using forward genetic studies. Arranged by chromosome, each locus has been drawn to scale based on the current mapping data for each. Putative loci or loci that lack mapping data have been excluded. Loci whose precise map location is unknown (indicated with a *) have been drawn centered over the peak marker of association. *Ccs*: Colon cancer susceptibility; *Cdcs*: Cytokine deficiency in colitis (*Il-10*^{-/-} mouse model of colitis); *Dssc*: Dextran sulfate sodium-induced colitis; *Gpdc*: G protein deficient colitis; *Hiccs*: *Helicobacter hepaticus*-induced colitis and associated cancer susceptibility; *Ibdq*: Inflammatory bowel disease quantitative trait loci (Spontaneous *SAMP1/YitFC* model of colitis); *Mom*: Modifier of min (*Apc*[Min+/-] model of CRC); *Scc*: Susceptibility to colon cancer; *Tm*: *Trishuris muris*-induced colitis; *Tnbs*: Trinitrobenzene sulfonic acid susceptibility.

we can increase the probability of identifying genetic factors specific to CA-CRC and not factors associated with increased colitis. Such loci can then be assessed in human cohorts, with the hope of identifying patients at high risk for colitis to CA-CRC transformation.

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