Gastrointestinal Cancer: Targets and Therapy

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REVIEW

New insights from animal models of colon cancer: inflammation control as a new facet on the tumor suppressor APC gem

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Abstract: Colorectal cancer (CRC) is one of the most common causes of cancer-related deaths worldwide. As with other cancers, CRC is a genetic disease, however, several risk factors including diet and chronic colitis predispose to the disease. Mutations in the tumor suppressor adenomatous polyposis coli (*APC*) initiate most cases of CRC. Recent data from mouse models suggest that *APC* mutations and colitis are not completely independent factors in colorectal carcinogenesis. Here, we review the evidence supporting an interaction between *APC* mutations and chronic colitis. We will also discuss possible pathophysiologic mechanisms behind this interaction.

Keywords: rodent model, colon cancer, adenomatous polyposis coli, APC, tumor suppressor, inflammatory bowel disease

Introduction

Colorectal cancer (CRC) is the fourth largest cancer killer worldwide and accounts for about 9% of cancer related deaths in the Unites States.[1] CRC is a genetic disease that results from accumulation of mutations in tumor suppressor genes and proto-oncogenes.[2] There are many factors that increase CRC risk, including age, diet, ethnic background, known genetic alterations, family history of the disease, and chronic colon inflammation (colitis).[3] Mouse and rat models developed to study CRC have confirmed some of the risk factors elucidated from human cases. These models also revealed many of the molecular events underlying different risk factors and interactions between various risk factors.[4,5] In this review we will discuss the interaction between the most common genetic alteration in CRC, mutations in the tumor suppressor *APC,* and a major predisposing factor for CRC, chronic colitis, as illuminated by studies of rodent models.

APC structure, functions, Wnt signaling

Mutations in *APC* are the most prevalent among genetic alterations found in CRC.[6] These *APC* mutations occur early during CRC tumorigenesis and are considered the initiating events of CRC.[2] In addition to the frequent somatic *APC* mutations, a more rare inheritance of a germline *APC* mutation in familial adenomatous polyposis (FAP) patients leads to development of tens to thousands of colonic adenomatous polyps.[7,8] Although benign, these polyps have, on average, a 1%–5% chance of undergoing malignant transformation. Considering the number of polyps that typically develop in FAP patients, CRC is nearly inevitable, unless the colon is surgically resected.[9]

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The *APC* gene encodes a large multidomain protein, 2,843 amino acids, that interacts with many other proteins and is implicated in multiple cellular processes.[10,11] The most characterized function of APC is to antagonize Wnt signaling-induced cellular proliferation by destroying the oncoprotein β-catenin.[12] APC is a component of a multiprotein cytoplasmic complex that phosphorylates and targets β-catenin for proteasome-mediated degradation. In the presence of Wnt ligand, or in the absence of functional APC, β-catenin accumulates in the cytoplasm and translocates to the nucleus, where it binds to the transcriptional cofactor TCF/LEF to alter the expression of Wnt target genes.[13] Most β-catenin-responsive genes are induced eg, *MYC*, *CyclinD1*, and *AXIN2*; and a minority are downregulated, eg, *HATH1*[14–17] (for an updated list of Wnt target genes see the Wnt homepage http://www.stanford.edu/group/nusselab/cgi-bin/wnt/target_genes).

Wnt signaling plays an important role in maintaining the intestinal epithelial architecture.[18] The intestine is lined by a single layer of columnar epithelial cells that are arranged in finger-like projections into the lumen (villi, only in the small intestine) and sac-like invaginations (crypts, in both the small and large intestines). Stromal cells at the crypt base secrete Wnt ligands that maintain a gradient Wnt concentration along the length of the crypt. Intestinal stem cells located at the crypt base (highest concentration of Wnt) divide to maintain the stem cell population and also produce progenitor transit amplifying cells (TA).[19] TA cells further divide until they reach the upper one-third of the crypt (with lower Wnt concentration) where they start to differentiate into various adult cell types.[13,20] The inability of mutant APC to antagonize Wnt signaling results in continuing proliferation, lack of differentiation, and intestinal tumor formation.[21–23]

Wnt-independent roles of APC include regulation of cellular adhesion, migration, cytoskeletal organization, spindle formation, cellular differentiation, and chromosome segregation.[10,24] APC coimmunoprecipitates with the adherens junction protein, β-catenin.[25,26] Full-length, but not truncated, APC colocalizes with microtubules and also concentrates near the leading edge of migrating epithelial cells.[27] This microtubule interaction involves the C-terminal part of APC and is unrelated to Wnt antagonism.[28] APC interacts with the microtubule-associated protein EB1[29,30] and with the intermediate filament proteins Lamin B1 and Keratin 81 in cultured cells.[31] Mutations in *APC* have been associated with chromosomal instability in both colon cancer cell lines and mouse embryonic stem cells.[24,32] Moreover, in mouse intestinal epithelial cells, *Apc* mutations affect the sensitivity of cultured cells to microtubule poisons, inhibiting spindle assembly checkpoint-induced mitotic arrest in response to low doses of microtubule poisons.[33]

In addition to the cytoplasmic functions described above, APC moves between the cytoplasm and the nucleus.[34–36] This nucleo–cytoplasmic shuttling is aided by two nuclear localization signals (NLS) in the C-terminal half of APC and five nuclear export signals.[36,37] Nuclear APC can antagonize Wnt signaling by sequestering nuclear β-catenin from interaction with the TCF/LEF transcription factor.[35,38]

Other proposed functions for nuclear APC include DNA synthesis, cell cycle regulation, and DNA repair.[36] APC interacts with Topoisomerase IIα, an enzyme essential in DNA replication and cell cycle progression.[39,40] APC also interacts with PCNA, FEN-1, and polymerase-β, components of long patch-base excision repair (LP-BER),[41–45] and affects CREB-C/EBP- mediated transcription.[46] Although the significance is not completely understood, APC appears to directly interact with A/T-rich DNA sequences.[47] It is important to note that cancer-associated mutations in *APC* usually result in deletion of the C-terminus of the protein, including several protein interaction domains and both NLS.[48]

Modeling Apc in rodents

To study APC biological functions in development and cancer, several mouse and rat models have been made. A more comprehensive review of these models are provided in other articles.[4,5] Most of these models have mutations resulting in truncated Apc, with lengths ranging from complete deletion to deletion of only the C-terminal 300 amino acids. Figure 1 shows protein products resulting from *Apc* mutations in rodent models that will be discussed in this review. These models displayed some of the same phenotypes as patients with germ line mutations of *APC*.[5] Mice with Apc truncation involving at least the C-terminal half of Apc develop intestinal tumors, though the number of tumors does not correlate with the extent of truncation.[4] As in FAP patients, *Apc* truncating mutations in these models are lethal in a homozygous state, and tumor development requires mutation or loss of the other (wild type) *Apc* allele.[5] Tumors from these mouse models resemble those found in patients at both the histological and molecular levels.[49] However, the mouse tumors mainly develop in small intestine, whereas FAP patients harbor mostly colonic tumors.[4] Rats with a mutation that truncates Apc at amino acid 1137 develop tumors in both the small and large intestine.[50] In addition, unlike in humans, progression to carcinoma is not typically seen in most *Apc* mutant mice, presumably because of their limited lifespan.[51] There are also

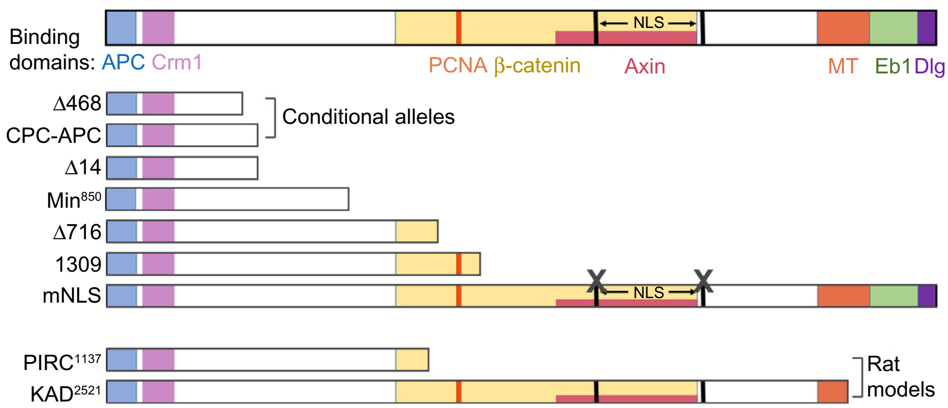

Figure 1 *Apc* mutant rodent models.

Notes: The 2,843 amino acid-long Apc protein with binding domains marked as follows: APC, homodimerization; Crm1/Exportin 1 binds NES to mediate nuclear export; PCNA (orange line), processivity factor for DNA polymerase δ; Eb1, binds and regulates MT plus ends. Shown below the schematic are the Apc proteins from rodent models referenced in this review.

Abbreviations: PCNA, proliferating cell nuclear antigen; MT, microtubule; Eb1, end binding 1; Dlg, discs large; NLS, nuclear localization signals.

some differences in the extraintestinal phenotypes in rodent *Apc* models and human FAP cases.[4,5] Recently, several new *Apc* rodent models have been generated to facilitate testing the function of a specific region or subcellular localization of Apc. These include a rat model with a shorter truncation (Apc KAD rat),[52] and mouse models with interstitial mutations deleting a specific Apc domain ($Apc^{\Delta SAMP}$)[53] or disrupting Apc nuclear localization signals (Apc^{mNLS}).[54]

In addition to rodent models with germ line *Apc* mutations, several models use LoxP-Cre technology to delete all, or portions, of Apc in a conditional manner.[5] In this system, deletion of a genomic region flanked by two *LoxP1* sites is induced by expression of Cre recombinase enzyme. Cre-mediated deletion is specified by placing *Cre* under the control of a tissue-specific, developmental stage-specific, or drug-inducible promoter, or by infecting the tissue with adenovirus that expresses Cre recombinase.[5] CPC–APC, Apc^{580D}, and $Apc^{\Delta 468}$ are three such models discussed further in this review (Figure 1).

Chronic colitis

Besides *APC* mutations, other factors such as chronic inflammation increase risk of CRC.[55] Inflammation is an immunological reaction to protect from harmful agents, including invading microorganisms.[56,57] An estimated 15% of all cancers are associated with chronic inflammation.[58] For the colon, patients with an inflammatory bowel disease (IBD, ulcerative colitis, or Crohn's disease) have 2–4 times increased risk of CRC compared to the general population.[55] This colitis-associated CRC is more aggressive and has a relatively poor prognosis.[59] Many inflammatory mediators have roles in the protumorigenic effects of IBD-associated inflammation.[55,59] These mediators are secreted by inflammatory as well as epithelial cells, and affect cellular survival, proliferation, apoptosis, and differentiation.[55,59]

Modeling chronic colitis in rodents

To facilitate studying colitis, a dextran sodium sulfate (DSS) model was developed in the rat and adapted to both hamster and mouse.[60–63] In this model, colonic inflammation is usually induced by administration of DSS (1%–4%) in drinking water for 3–7 days. Mice are then typically given untreated water for 2–4 weeks, with the cycle repeated up to four times.[61]

The DSS model appears similar to human ulcerative colitis at both the pathological and molecular levels.[64] The pathological changes seen during the first DSS cycle in murine colons include loss of crypt structure and ulceration, symptoms that are also seen in the acute phase of the human disease.[65] Following the first cycle, mucosal regeneration, crypt branching and shortening, glandular disorder, and diarrhea are also seen; these also occur in the chronic phase of ulcerative colitis in humans. As with human IBD, mice treated with DSS also show an increased incidence of colonic tumors that varies somewhat based on the protocol of DSS treatment.[65,66] For Swiss mice treated with four cycles (7 days each) of 4% DSS, the colon tumor incidence is about 37.5% at 120 days and more than half of the lesions that develop in DSS-treated mice are flat, similar to those seen in the human disease.[66] Some tumors in this model show malignant transformation.[66] Molecular changes in tumors from DSS-treated mice also recapitulate those in human colitis-associated colorectal carcinogenesis.[66,67]

Administration of a mutagen increases the incidence of colonic tumors in the murine DSS model.[64] The most commonly used mutagen is azoxymethane (AOM), which induces O[6]-methylguanine DNA adducts resulting in G→A transitions.[64] A single intraperitoneal dose of AOM increases the incidence of colonic cancer in DSS-treated mice to 100%.[64] Another advantage of including a mutagen in the protocol is that it allows reduction of the DSS dose in mice, and decreases the mortality from DSS-associated acute colitis. Again, different groups use different regimens of AOM treatment: single or multiple doses of 7.5–20 mg/kg. A single AOM dose of 10 mg/kg alone without DSS treatment is not sufficient to induce tumors in wild-type mice.[64]

β-catenin mutations in exon 3 are detected in most tumors from AOM–DSS-treated mice.[68] These mutations are expected to prevent phosphorylation and targeting of β-catenin for destruction, resulting in cellular accumulation and nuclear translocation of β-catenin, and promiscuous activation of Wnt signaling.[68] On the other hand, many AOM-induced tumors in rats have *Apc* mutations.[69] Both mice and rats treated with AOM–DSS have activating mutations of the proto-oncogene, *Kras,* in later stage tumors.[68] Wnt and RAS pathways are typically activated in human CRC.[2]

Intestinal epithelial barrier and gut microbiome

Colon epithelial cells are exposed to a unique external environment. The colon lumen contains hard fecal matter, posing a potential threat of mechanical injury.[70] In addition, the colon is inhabited by over one hundred trillion bacterial cells (almost ten times the number of cells in an adult human). These gut microbes consume organic materials and secrete various secondary metabolites.[71,72] Intestinal epithelial cells have several lines of defense that prevent bacterial invasion or diffusion of harmful substances into the body while allowing absorption of nutrients and beneficial substances.[73] These combined structural and physiological defenses are termed the "intestinal epithelial barrier".[70,74]

There are at least seven contributors to the intestinal epithelial barrier (Figure 2). First is the actual physical barrier created by mucus, which is continuously secreted by goblet cells.[75] This mucus is formed of two layers; an outer loose layer and an inner adherent layer. The outer mucus lubricates the solid contents of the colon to prevent mechanical injury and also washes off microorganisms to prevent colonization.

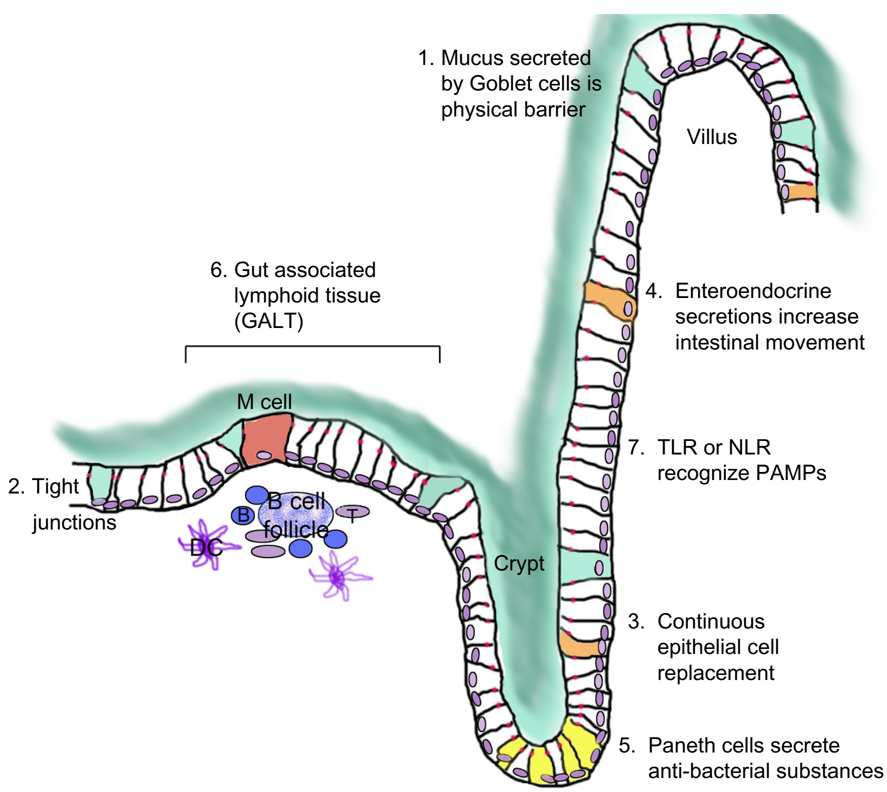

Figure 2 Intestinal epithelial barrier.

Notes: Protecting the body from invasion by intestinal microbes requires many layers of defense. This illustration depicts the small intestine. The colon would have similar components but lack Paneth cells and the villus structure. Goblet cells (green); tight junctions (red); enteroendocrine cells (orange); Paneth cells (yellow).

Abbreviations: DC, dendritic cell; T, T-cell; B, B-cell; TLR, Toll-like receptors; NLR, Nod-like receptors; PAMP, pathogen-associated molecular patterns.

The inner mucus layer prevents contact of microorganisms and their products with the underlying epithelial cells.[75] Second, epithelial cells lining the colon form a continuous sheet with tight junctions that further prevent flora and harmful molecules from penetration.[76] Third, the continuous turnover of intestinal epithelial cells ensures rapid healing after any damage or ulceration.[70,77] Fourth, specialized epithelial cells, enteroendocrine cells, respond to bacterial invasion or toxic substances by secreting active amines to increase intestinal movement and fluid secretion, thereby washing off potential invaders.[78] Fifth, in the small intestine, other specialized epithelial cells called Paneth cells secrete antibacterial substances. Sixth, intestinal tissue also contains aggregations of immune cells (gut-associated lymphoid tissues [GALT] and other immune cells) that can detect foreign antigens and defend the body against them. M-cells also contribute by engulfing antigens and bacteria from the lumen and transporting them to antigen presenting cells for immunological processing.[70] Seventh, intestinal epithelial cells themselves detect different microbes and react to them by expressing receptors that can recognize pathogen-associated molecular patterns (PAMP) including Toll-like receptors (TLR) and Nod-like receptors (NLR). These receptors do not recognize specific antigens but specific molecular signatures associated with pathogens eg, methylated DNA and peptidoglycans.[79]

Colitis and APC mutations

CRC is fundamentally a genetic disease, the result of accumulated mutations in tumor suppressor genes and oncogenes.[2] But the nature of the mutated genes and the order of their mutation can vary with different precipitating factors.[67,80,81] Activation of Wnt signaling is seen in the vast majority of CRCs.[2] Other signaling pathways that are commonly altered during CRC progression include activation of K-ras, p53, and TGF-β.[82] Alterations in the same pathways are frequently seen in cases with colitis-associated CRC. In addition, activation of NF-κB and STAT3 pathways are also detected in colitis-associated CRC. The sequence and role of these pathway alterations in the development of CRC have been reviewed previously.[59] Here, we will focus on genetic mutations of the tumor suppressor *APC*.

Mutation of *APC* is by far the most common genetic event seen in CRC that leads to Wnt signal activation. Curiously, *APC* mutations are not detected in other Wnt-dependent tumors to nearly the same extent as seen in CRC. Rather, in non-colonic tumors, mutations in other Wnt components, are more commonly found,[12] suggesting a colon-specific protective function of APC that is selected against during CRC development. Furthermore, data from AOM–DSS models suggest that Wnt signal activation alone is not sufficient for effective initiation of colon tumorigenesis. Injection of mice with a single dose of AOM, expected to induce oncogenic β-catenin mutations which activate Wnt signaling, results in no tumors or only a very low incidence of tumors.[61,68] However, combing AOM with DSS-induced inflammation results in robust tumor formation. Moreover, patients and mice with germ line *APC*/*Apc* mutations develop intestinal tumors with 100% penetrance.[5,9]

The data supporting an association between *APC* mutations and inflammation are overwhelming. Inflammation can greatly increase intestinal tumorigenesis in rodent models with germ line *Apc* mutations. DSS treatment of $Apc^{Min/+}$ mice increases their colon tumor multiplicity by 15–30-fold.[83] Unlike AOM-induced tumors in wild-type mice treated with DSS, which show β-catenin stabilizing mutations, colonic tumors in DSS-treated $Apc^{Min/+}$ mice typically show loss of the wild-type *Apc* allele.[83] The latter mechanism is similar to that seen in tumors from $Apc^{Min/+}$ mice not treated with DSS.[84] Of note, the multiplicity of tumors in DSS-treated $Apc^{Min/+}$ mice is higher than in wild-type mice treated with the mutagen AOM followed by DSS.[83] Collectively, these data strongly support a colon-specific tumor suppressor function for APC beyond that as a Wnt signal antagonist, potentially to control colitis.

Experimental induction of inflammation in mouse intestinal tumor models by methods other than DSS administration also increases tumorigenesis. Germ line deletion of *Il-10* (an anti-inflammatory cytokines) or single immunoglobulin Il-1 receptor-related (*SIGIRR*) molecule increases intestinal tumors in $Apc^{Min/+}$ mice.[85,86] Transgenic expression of *Il-8* (a proinflammatory cytokine) enhances tumorigenesis in both AOM–DSS and $Apc^{Min/+}$ models.[87] In addition, *Nrf2* knockout mice display increased oxidative stress, increased inflammatory markers, and colitis and accelerated intestinal tumorigenesis.[88,89] Conversely, reducing inflammation protects from intestinal tumorigenesis. Nonsteroidal anti-inflammatory drugs (NSAIDs) reduce polyp formation in FAP patients as well as in $Apc^{Min/+}$, $Apc^{\Delta474/+}$, and $Apc^{1309/+}$ mouse models.[90–94] Experimental genetic deletion of proinflammatory mediators *CXCR2, CD24, TNF-α*, and *epimorphin* significantly reduces intestinal tumor numbers in $Apc^{Min/+}$ mice.[95–98]

Inflammation might also contribute to some other known risk and protective factors in CRC. For example, high fat diets and obesity predispose humans to CRC, $Apc^{Min/+}$ mice to increased intestinal polyposis, and AOM-treated mice to pre-cancerous colon lesions.[99,100] Obesity has been associated with adipose tissue macrophage malfunction and low-level inflammation.[101–103] A recent report showed increased inflammatory mediators in *Apc*$^{Min/+}$ mice on high fat diet relative to *Apc*$^{Min/+}$ mice on regular lab diet.[104] In addition, many natural products including curcumin, grape antioxidant fibers, and brown rice reduce colon tumors in various mouse models, presumably by reducing inflammation.[105–109]

The mechanisms by which inflammation can enhance colon tumorigenesis are not completely delineated. Inflammation activates many pathways that synergize with Wnt signal activation in CRC tumorigenesis including AKT, KRAS, BRAF, HIF1-α, and TGF-β. DNA damage and epigenetic changes that are associated with inflammation could also contribute to tumor formation.[59]

Many inflammatory pathways converge to activate the prosurvival NF-κB pathway,[59] which is also activated in colonic mucosa from IBD patients.[110] NF-κB pathway activation increases proliferation and decreases apoptosis in CRC cell lines and mouse colon mucosa,[110,111] drugs that inhibit the NF-κB pathway decrease intestinal tumorigenesis in *Apc*$^{Min/+}$ mice.[112] Aspirin, an NSAID that decreases intestinal polyposis in both mouse models and FAP patients and protects from CRC, inhibits the NF-κB pathway and also increases *Apc/APC* expression.[113]

Inflammation can increase DNA damage and accelerate mutagenesis. The rate of reactive oxygen species (ROS) production, including nitric oxide (NO), is augmented in inflamed tissues. ROS are genotoxic and increase DNA mutation rates.[55,58,114,115] Inhibiting NO production reduces intestinal polyp formation in *Apc*$^{Min/+}$ mice as well as inflammatory models of colitis.[116,117] Notably, activation of the NF-κB pathway by constitutive activation of its upstream activator, IKKβ, enhances intestinal polyposis and elevates DNA damage in *Apc*$^{580D/+}$ mice.[118] NO synthase inhibitors reduce this DNA damage and intestinal tumorigenesis, suggesting that accelerating *Apc* LOH (loss of heterozygosity) due to the DNA damaging effect of NO is the cause of enhanced tumorigenicity in these mice.[118] Inflammation may also induce DNA damage by increasing the production of other mutagenic factors including trans-4-hydroxy-2-nonenal from the activated inflammatory cells, which can further induce chromosomal breakage in nearby epithelial cells.[119] Moreover, chronic inflammation can also reduce DNA mismatch repair proteins.[120,121]

Chronic inflammation is also associated with epigenetic changes including changes in miRNA, DNA hypermethylation, and aberrant methyl histone markings.[122] Colitis leads to upregulation of *miRNA-155*[123]; *miRNA-155* targets *APC* and thus, activates β-catenin.[124] The protumorigenic effect of chronic colitis has also been linked to prostaglandin (PG) formation through induction of cyclooxygenase-2 (COX-2).[125] COX-2 is the rate-limiting step in PGE2 formation from arachidonic acid.[122] Both Cox-2 and PGE2 promote Wnt signaling, increase cellular proliferation, inhibit apoptosis, promote angiogenesis, and enhance metastasis.[126–129] Conditional deletion of *Cox-2* results in significant reduction of intestinal tumors in *Apc*$^{Min/+}$ and *Apc*$^{\Delta716/+}$ mice,[130,131] Cox-2 is also targeted by NSAIDs and selective Cox-2 inhibitors such as Celebrex, both of which reduce intestinal tumorigenesis in patients and mouse models with germ line *APC*/*Apc* mutations.[122]

APC mutations and inflammation

In the previous section we presented evidence that inflammation accelerates intestinal tumorigenesis in the presence of *Apc* mutations. However, there is evidence that *Apc* mutations can enhance colitis. Proinflammatory mediators *Cox-1*, *Cox-2, MIP-2, OPN, CXCR-2*, and *Gro-α* mRNA are upregulated in colonic polyps in *Apc*$^{Min/+}$ mice relative to epithelial cells from normal mice.[132] Of these genes, only *Cox-2* is a defined Wnt target.[133,134] The other mediators have not been linked to activated Wnt signaling resulting from *Apc* mutations. In addition, mRNA and serum protein levels of proinflammatory cytokines MCP-1, IL-6, IL-1β, and TNF-α increase with the progression of intestinal tumorigenesis and correlate with tumor size.[135] Moreover, a global expression analysis showed differential expression of inflammatory genes, *Lcn2* and *N4wbp4*, in *Apc*$^{Min/+}$ polyps.[136] In another mouse model (CPC–APC), conditional truncation of *Apc* in the distal part of the small intestine and colon resulted in inflammatory cell infiltration and upregulation of Il-17 and Il-23 in the developing polyps.[137]

Recently, we described a mouse model with a germ line *Apc* mutation that compromises the ability of Apc to locate to the nucleus.[54] These *Apc*$^{mNLS/mNLS}$ mice only rarely develop tumors, and homozygous mutant mice are viable. However, the *Apc*^{mNLS} allele increases tumor formation when combined with the *Apc*^{Min} allele (*Apc*$^{mNLS/Min}$ mice).[54] Notably, *Apc*$^{mNLS/mNLS}$ mice have higher expression of inflammatory mediators *Cox-2* and *MIP-2* and are more susceptible to DSS-induced colitis and AOM–DSS-induced colon tumorigenesis.[138] Rats with germ line *Apc* mutation resulting in truncation of the C-terminal 300 amino acids (KAD rats) do not develop tumors but are also more susceptible to DSS-induced inflammation and AOM–DSS-induced colon tumorigenesis.[139]

APC mutation can induce colitis by several mechanisms. First, *APC* mutations can decrease mucus production and therefore reduce the barrier between gut microbes and intestinal tissues.[137] Apc normally functions in promoting cellular differentiation of intestinal lineages including mucus-producing goblet cells.[23,140] *Apc*$^{mNLS/mNLS}$ mice have reduced expression of *Hath-1* and fewer goblet cells in their small intestines and less *Muc-2* mRNA in their colons, relative to their wild-type littermates.[54,138] Hath-1 is a transcription factor that participates in goblet cell differentiation and is negatively regulated by Wnt signaling.[17,141] Muc-2 is the major protective mucin in the colon. *Muc-2* knockout mice develop colitis and have spontaneous colonic tumors.[142,143] *Muc-2* mutation also enhances intestinal tumorigenesis in *Apc*$^{Min/+}$ mice.[143,144] Furthermore, induction of inflammation in *Apc*$^{mNLS/mNLS}$ mice using DSS results in significantly fewer goblet cells and reduced *Muc-2* mRNA, relative to DSS-treated wild-type mice.[138] Goblet cell differentiation requires low Notch signal and treating *Apc*$^{Min/+}$ mice with a γ-secretase inhibitor, inhibited Notch signaling and increased goblet cell differentiation in intestinal tumors.[145] A potential link between Notch signaling and APC is that APC is in a double negative feedback loop with the transcription inhibitor Msi-1.[146] Msi-1 activates Notch signaling by inhibiting the Notch repressor, Numb.[147] In cases of *Apc* mutation, Msi-1 is upregulated; activating Notch signaling.[23,148] However, a direct role of Msi-1 in goblet cell differentiation has not been examined. Finally, FAP patients and CPC–APC mice with conditional truncation of APC/Apc showed reduced mucus production of polyps, which displayed *Apc* LOH.[137] Colonic mucosa in AOM-treated rats as well as FAP patients shows foci with depleted mucin.[149,150] These mucin-depleted foci are correlated with tumor number and have high rates of *Apc* mutations.[151] *Apc*-mutant (PIRC) rats also show mucin-depleted foci that increase in number as the rats age.[152] Notably, the NSAID sulindac, reduces the number of polyps as well as mucin-depleted foci in PIRC rats.[152] Collectively, these data suggest that Apc mutations predispose to the precancerous mucin-depleted foci.

Alteration of *Apc* can also affect other intestinal epithelial barrier activities. APC loss effects localization of tight junction protein ZO-1.[153] Loss of APC and upregulated Wnt signaling are also associated with increased expression of tight junction protein claudin-1 in CRCs.[154] Further, inducible Apc truncation in CPC–APC mice leads to reduced junctional claudin-3, -4, -5, and -7 and decreased levels of JAM-C (junctional adhesion molecule-C) mRNA.[137] The C-terminus of Apc binds to the junctional protein DLG (Figure 1). In KAD rats, Dlg5 fails to localize to the junction in endothelial cells, resulting in delayed healing after DSS-induced inflammation.[155] Finally, APC interacts with cytoskeletal proteins including those of microtubules and intermediate filaments, which are important in formation and maintenance of tight junctions.[31,156,157] *Apc* mutations alter cytoskeletal organization in intestinal epithelial cells and affect cell polarity.[158] Whether these changes in epithelial organization enhance colitis is not clear.

Apc mutations might also induce inflammation by activating Wnt signaling. *Cox-2* and *iNOS* are Wnt targets.[134,159] Cox-2 is the rate-limiting enzyme in PGE2 synthesis. PGE2 is involved in processes that lead to inflammation, including, vasodilation, increasing vascular permeability, and chemoattraction of inflammatory cells.[59]

APC, colitis, and microbiome in CRC

The role of intestinal flora in health and disease is getting increasing attention of late.[160,161] The development of tools such as deep sequencing has allowed rapid analysis of different intestinal bacteria. The gastrointestinal tract in general and especially the distal portion is home to a large number of microorganisms. The relationship between these florae and the host is mostly symbiotic.[160,161] The host provides a niche and nutrients, while intestinal florae provide essential vitamins and are crucial for the development of the host immune system. Particular intestinal florae also prevent overgrowth of pathogenic microorganisms by competing with them for limited resources. However, changes in the number, type, or the relative abundance of different intestinal microorganisms (dysbiosis) have been related to many pathological conditions including IBD and CRC.[160,162] The challenging task for the intestinal epithelial barrier is to regulate the intestinal microbiome by allowing the growth of beneficial species and preventing the growth and invasion of pathogenic and opportunistic organisms.

Disruption of the intestinal epithelial barrier is a hallmark of IBD.[163] However, the relationship between the epithelial barrier, intestinal florae, and inflammation has multiple levels of complexity. Mucus secretion is stimulated by bacterial colonization.[164] Germ-free mice have a thin mucus layer, which can be restored to normal thickness by bacterial products including peptidoglycans and lipopolysaccharides.[164,165] Bacterial products including butyrate and short chain fatty acids also can induce *Muc2* transcription via c-Fos/c-Jun and by epigenetic histone alterations.[166–169] On the other hand, microbes or their metabolic products may induce inflammatory reactions in the colon. Some intestinal florae such as *Fusobacteria* and *Surpulina* are enriched in the mucus layer covering regions of enteric inflammation,[170,171] consistent with their ability to dissolve the mucus layer and thus provide access to other microbes.[172] *Clostridia*-like gram-positive segmented filamentous bacteria induce intestinal inflammation which predisposes to colitis but also protects mice from some enteric infections.[173] In contrast, some bacterial products such as short chain fatty acids and butyrate inhibit colitis by stimulating epithelial cells to secrete the anti-inflammatory cytokines IL-10 and IL-18.[174–176]

Several mechanisms linking the colonic microbiome to CRC have been proposed. In human patients, the florae of colonic adenomas and adenocarcinomas are enriched with fusobacterial species relative to normal colon tissue.[177,178] *Fusobacteria* enhance intestinal tumorigenesis in *Apc*$^{Min/+}$ mice resulting in a proinflammatory gene expression signature in the tumor cells.[177] Reducing microbial-induced inflammation by deleting the PAMP pathway adaptor protein Myd88 decreases intestinal tumors in *Apc*$^{Min/+}$ mice and colon tumors in AOM-treated mice.[179,180] Furthermore, transplantation of bone marrow from mice with mutations in genes encoding PAMP adaptor proteins Myd88, Tlr2, 4, and 9 reduces inflammation and tumor load in CPC–APC mice.[137] Finally, deletion of anti-inflammatory cytokine *Il-10* alters the intestinal microbiota and increases the intestinal tumor number in *Apc*$^{\Delta 468}$ mice; treating these mice with broad-spectrum antibiotics decreased the overall microbial diversity and also decreased the intestinal tumor multiplicity.[181]

Microorganisms can also secrete carcinogenic metabolites that can mutate DNA. In addition to ROS produced by inflammatory cells as the result of bacterial-induced inflammation, some colonic bacteria including the gram-positive *Enterococcus faecalis* produce hydroxyl radicals.[182–184] Still, other colon-inhabitant gram-negative, *Escherichia coli*, produce a toxin that can cause DNA damage and CRC.[185] Bacteria may also secrete chemicals that directly induce proliferation. For example, the exotoxin fragilysin secreted by some *Bacteroid* species induces c-Myc which stimulates cellular proliferation.[186] Bacterial metabolites such as H_2S are produced by many *Enterobacterial* species commonly found in the normal colon.[187] H_2S can activate the RAS-MEK pathway and induce cellular proliferation in mice.[188]

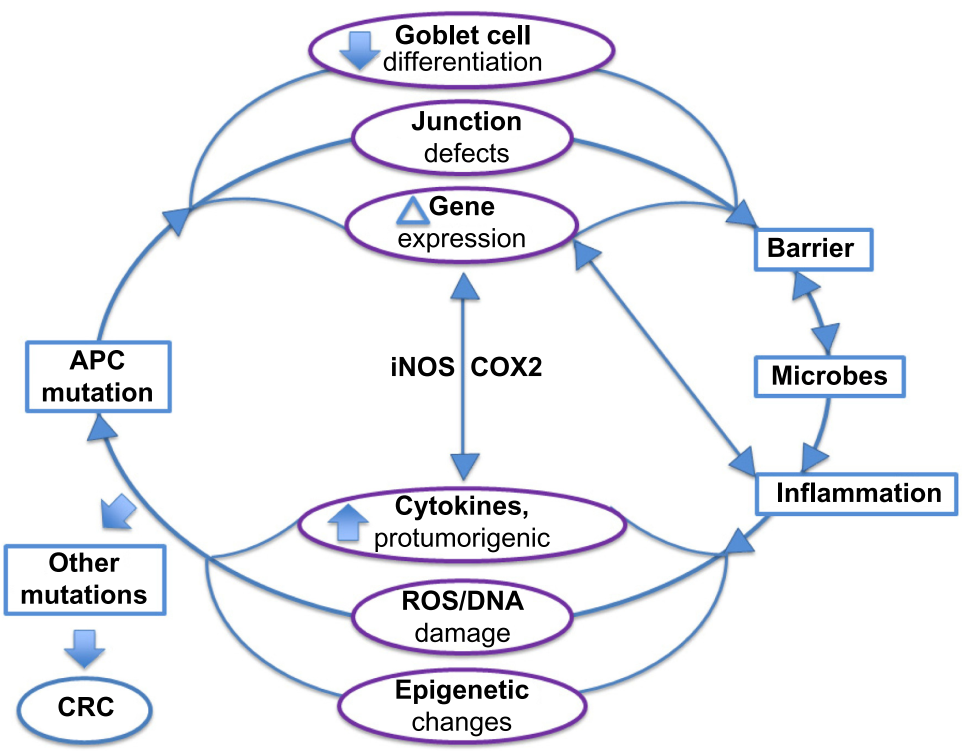

Figure 3 Potential roles for APC in inflammation.

Notes: APC normally promotes differentiation of goblet cells which generate and secrete mucus. Protective mucus layers provide a physical barrier between luminal microbes and the epithelial cells lining the intestine. APC interacts with various junctional proteins, further contributing to a barrier between the luminal contents and the immune cells of the stroma. APC regulates expression of genes, some of which are involved in inflammation. Microbial breach of the intestinal barrier results in inflammation. Consequences of inflammation include DNA damage and epigenetic changes that can result in additional mutation of tumor suppressor genes and oncogenes that further promote colorectal carcinogenesis.

Abbreviations: APC, adenomatous polyposis coli; iNOS, induced nitric oxide synthase; CRC, colorectal cancer; ROS, reactive oxygen species; COX-2, cyclooxygenase-2.

Several observations made in mouse models point to an interaction between genetic lesions, intestinal florae and CRC. Smad3-deficient mice develop colon tumors only in the presence of helicobacter infection.[189] *Tbx2* and *Rag2*$^{-/-}$ ulcerative colitis (TRUC) mice develop colitis and colitis-associated colon cancer, but not when raised in a germ-free environment.[190] Similarly, *Il10*$^{-/-}$ mice develop colitis-associated colon tumors only if they have intestinal bacteria.[191] NLRP6 is a component of the innate immune response that senses microbes, and NLRP6 deletion in intestinal epithelial cells induces colitis and colitis-associated tumorigenesis.[192] These NLRP6-deficient mice also have changes in the bacterial flora composition with more abundant *Bacteroids* in the colon. Remarkably, cohousing these *NLRP6*-mutant mice with wild-type mice results in development of colitis and colon tumors in the wild-type mice, consistent with transmissible tumor promoter.[192,193] A similar transmissible, tumor-promoter has been described in mice with mutations in other components of the innate immune response, NOD2 and RIP2.[194] Furthermore, expression of the secreted anti-inflammation mediator/antimicrobial, *Pla2g2a* in intestinal epithelial cells reduces the incidence of intestinal polyps in *Apc*$^{Min/+}$ mice and in orthotopic xenografts of human colon cancer cells.[195,196] Notably, exogenous expression of the *Pla2g2a* gene prevents colon tumorigenesis in Muc2-deficient mice.[197]

Although connections are starting to emerge, the precise relationship between the tumor suppressor Apc and intestinal flora is not well defined. *Apc*$^{Min/+}$ mice raised in a germ-free environment develop fewer polyps than *Apc*$^{Min/+}$ mice housed in standard conditions.[198] However, this tumor reduction is statistically significant only in the middle portion of the small intestine, with no reduction in the number of tumors in the colon.[198] This region specificity may represent a varied role for different microbial species in discrete regions of the gastrointestinal tract. On the other hand, *Apc*$^{\Delta 14/+}$ mice developed more polyps when raised in germ-free conditions than in standard housing conditions.[199] Together, these data suggest an allele-specific interaction of *Apc* with the microbial content of the gut. Notably, mutations in *Apc*$^{Min/+}$ and *Apc*$^{\Delta 14/+}$ are expected to result in truncated Apc proteins that differ by 403 amino acids.[5] The contrasting effect of germ-free conditions on polyp number in *Apc*$^{Min/+}$ and *Apc*$^{\Delta 14/+}$could also represent other contributing factors that vary between the two experimental conditions including other genetic loci and diet.[4]

Conclusion

The results gathered from studies of rodent CRC models reveal a complex interplay of genetics, inflammation, and the microbiome that gives rise to a cancer phenotype. APC is a major tumor suppressor in the colon. Although the most universally appreciated APC role is that of Wnt signal antagonist, APC is multifaceted. In this review, we describe an emerging role for APC in colitis. We propose that this APC role as regulator of the inflammatory response might be particularly critical in the colon and thus contribute to the high frequency of *APC* mutations seen in CRC compared to cancers of other tissues (Figure 3). Unearthing the precise role for APC in suppression of inflammation will expand the repertoire of therapeutic strategies aimed at rescuing the functions of this multifaceted and fascinating tumor suppressor protein.

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Disclosure

The authors report no conflicts of interest in this work.

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