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Review

Mouse Models of Inflammatory Bowel Disease - Insights into the Mechanisms of Inflammation-associated Colorectal Cancer

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Abstract. *The association between chronic inflammation and cancer has been noted for at least a century but the exact molecular mechanisms of cancer initiation and promotion by such inflammation are still poorly understood. The gastrointestinal tract is a unique organ where maintaining a balance between the colonic epithelial cells, the immune system and a fine-tuned response to the resident microflora is crucial for preserving the gut homeostasis. A breakdown of the tight interdependent regulation of the epithelium–immunity–microbiota triangle leads to inflammatory bowel disorders and may promote cancer. This review focuses on inflammation-associated colorectal cancer in mouse models of the disease and highlights emerging research trends.*

Inflammation-associated Colorectal Cancer

Inflammatory bowel disease (IBD) is the collective name for a group of gastrointestinal chronic inflammatory disorders with two major types of clinical presentations: ulcerative colitis (UC) and Crohn's disease (CD). IBD is an idiopathic disorder but it is now accepted that both environmental and genetic factors contribute to the pathogenesis of IBD. The molecular events leading to a breakdown in intestinal homeostasis and driving IBD pathogenesis include dysregulation of the innate and adaptive immune responses, loss of immune tolerance to the commensal microflora and a disruption of the intestinal epithelial integrity (1, 2). There is regional heterogeneity in the incidence of UC and CD worldwide and IBD disorders affect an estimated 2.4 million people in Europe alone and 1.3 million people in the USA (3). Colorectal cancer (CRC) is the cause of approximately 15% of all deaths in patients suffering from IBD (4).

UC affects the colon in a continuous fashion and always involves the rectum in adults, while CD can affect any part of the gastrointestinal tract, as intermittent lesions, but most commonly the terminal ileum or the perianal region (5, 6). Generally in UC the inflammation is superficial and restricted to the mucosa, whereas in CD the inflammation is often transmural and the mucosa appears thickened. In addition, granulomas, abscesses, fistulas and strictures are common features of CD. The disease location is relatively stable in patients with CD but the phenotype changes from non-stricturing and non-penetrating to either stricturing or penetrating over the course of the disease (7).

Patients with IBD have an increased risk of developing CRC, most of which is thought to be due to the persistent inflammatory response rather than a genetic predisposition (8). The risk of developing CRC increases with disease duration and extent, early onset, presence of primary sclerosing cholangitis (chronic inflammation of the bile ducts) and a family history of sporadic CRC (9-12). Although there is a clear association between chronic inflammation and cancer risk, the exact molecular mechanisms responsible for this increased risk are still poorly understood.

Molecular Alterations in Inflammation-associated Colorectal Cancer

In sporadic CRC the progression from normal epithelium to cancer involves the development of an adenomatous polyp (adenoma) precursor, which can be removed by endoscopic polypectomy. In contrast, in IBD-associated cancer, the precursor of neoplasia is usually a flat dysplastic lesion, and therefore its detection and removal is difficult (8).

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Inflammation-associated and sporadic CRC share similar molecular features: chromosomal instability (CIN), microsatellite instability (MSI) and CpG island methylator phenotype (CIMP), but their timing and frequency may differ. For example, in contrast to sporadic CRC, *p53* inactivation is an early event in IBD-associated cancer, while mutations of v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) and adenomatous polyposis coli (*APC*) are infrequent and occur later (13-15). High-level MSI (MSI-H), resulting from deficient mismatch repair, is found in 15% of neoplastic lesions in IBD, which is similar to sporadic CRC. However, low-level MSI (MSI-L) may be more frequent in IBD-associated neoplastic and non-neoplastic tissues due to chronic inflammation (16-18).

Methylation silencing of tumour suppressor genes is an important feature of sporadic CRC. CIMP is characterized by concordant methylation of cancer-specific genes and long-range epigenetic silencing involves large genomic regions, such as the entire 4-Mb band of chromosome 2q.14.2 (19, 20). In addition to CpG islands and gene promoters, methylation is found at sequences up to 2 kb distant from CpG islands, termed CpG island shores (21). Methylation also occurs in IBD-related cancer and a high degree of age-related methylation has been found in the dysplastic and inflamed mucosa of patients with UC, indicating that methylation of CpG islands precedes dysplasia (22). Frequent and early promoter hypermethylation has been found in UC dysplasia and neoplasias for a number of genes including genes frequently altered in sporadic CRC such as $p14^{ARF}$ and $p16^{INK4A}$. However, not all reports support an association between increased CpG island promoter methylation and IBD-related cancer (23-26).

Sporadic colorectal tumours arising in the proximal or distal colon may differ significantly in their molecular, clinical and histopathological characteristics, and the progression model (27, 28). CIN tumours are more frequently located in the distal colon and rectum, whereas, MSI tumours are predominantly located in the proximal colon and are associated with mucinous histology, poor differentiation and lymphocytic infiltration (29-31). CIMP tumours are characterized by a distinct set of features such as proximal colon location, v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) mutations and MSI-H (32-34). There is limited information about the tumour location prevalence of inflammation-associated CRC. One study examined the spatial distribution of CRC in 3,124 patients with CD and 3,093 patients with UC (35). The majority of carcinomas developed after 8 years of disease (CD 75%; UC 90%) in the areas of macroscopic disease. The anatomical distribution of cancer in the two diseases was significantly different. Tumours were located in the proximal colon in 49% of patients with CD and in 36% of patients with UC. Remarkably, the clinicopathological features of the tumours developing in CD and in UC were strikingly similar, including proportions of mucinous and signet ring carcinomas (35).

Mouse models of IBD have given some insights into the key factors and processes that contribute to colorectal tumour initiation and growth in the context of chronic inflammation. Here we review how the main mouse models of IBD and cancer (Table I) highlight the complex interactions between colonic epithelial cells, the immune system and the colonic microbiota, which are dysregulated in carcinogenesis.

The Role of the Colonic Epithelial Barrier in Maintaining Gut Health

Disruption of the epithelial barrier function can lead to impaired mucosal integrity, chronic inflammation and carcinogenesis, which has been exploited in many mouse models of IBD and cancer. In the healthy intestine there is a close spatial localization and a well-orchestrated crosstalk between cells of the mucosal epithelial barrier and cells of the mucosal innate and adaptive immune system. In addition, gut epithelial and dendritic cells (DCs) are in constant communication with the intestinal microbiota and more importantly, sensing of the commensal bacteria in normal conditions is essential for the maintenance of mucosal integrity and homeostasis (36). Because of this close spatio-functional relationship with the resident microbiota, the intestinal immune system functions in a tolerogenic state, which maintains tolerance towards the commensal microflora and harmless food components. For example, intestinal mucosal DCs preferentially induce differentiation of T-cells towards T-helper type 2 (Th2) and T-regulatory (Treg) subsets with tolerogenic properties (37, 38).

The colonic epithelial barrier consists of a monolayer of cells with intercellular tight junctions, and biochemical adaptations, which serves as an additional level of defence against the luminal microbiota. One of these adaptations is the glycosylated mucin-rich layer, forming a thick, impermeable sheet on the apical surface of intestinal epithelial cells (IECs). IECs also secrete a broad range of peptides with antimicrobial properties, such as defensins, cathelicidins and calprotectins (39, 40). Increasing evidence suggests that the epithelial layer not only serves as a passive physical barrier between the host and intestinal microflora, but is a dynamic, active participant in sensing external or endogenous 'danger' signals and effectively mounting immune responses (41).

IECs can recognise 'danger' signals. Microbial molecules, cytokines, and pro-inflammatory lipids signal infection and trigger an inflammatory response. An inflammatory response can also be initiated by tissue injury signals (heat-shock proteins, neuropeptides, mitochondrial peptides) (42). Stimulation of human colonic epithelial cells with pathogenic

bacteria and cytokines results in up-regulation of a distinct array of pro-inflammatory cytokines such as tumour necrosis factor alpha (TNFα), interleukin-8 (IL-8), monocyte chemotactic protein-1 (MCP-1), and granulocyte macrophage colony-stimulating factor (GM-CSF) (43). Once the inflammatory response has been initiated, efficient resolution of inflammation is important in order to minimize tissue injury and restore the integrity of the mucosal barrier. IECs play an active role in the resolution phase of inflammation by secreting anti-inflammatory mediators that generally inhibit neutrophil function. For example, anti-inflammatory lipoxins, derived from IECs inhibit neutrophil migration and stimulate neutrophil apoptosis and macrophage-mediated scavenging of neutrophils (44). Importantly, lipoxins inhibit the production of IEC-derived IL-8, a potent neutrophil chemoattractant (45).

Deficiency in the core 3-derived O-glycans, a major component of the intestinal mucus, results in susceptibility to dextran sodium sulfate (DSS)-induced colitis and tumourigenesis in mice (46). Altered O-glycans are a feature of UC and are detected in more than 90% of UC-associated CRC (47, 48). Compromised integrity of the epithelial barrier allows direct contact of the intestinal mucosa with the resident colonic bacteria and plays a crucial role in the development of inflammation. Other defects that lead to colitis due to increased intestinal permeability include altered intercellular adhesion (N-cadherin transgenic mice), and deficiency in mucosal structural and protective proteins such as keratin-8 and MUC2 (49-52). MUC2 is the main secretory mucin in the mucus layer. Mice deficient in MUC2 exhibit spontaneous colitis, epithelial hyperproliferation, loss of crypt architecture and develop invasive adenocarcinomas in the small bowel, as well as in the rectum. Similarly, chemically-induced disruption of the colonic epithelium integrity with DSS leads to mucosal ulceration and dysplasia even in wild-type mice (53, 54).

Mice with Dysregulation of the Immunosuppressive IL-10 or Transforming Growth Factor beta (TGF-β) Pathways

Under normal conditions, IECs serve as communicators between the commensal bacteria and the host immune system and such cross-talk is essential for the maintenance of epithelial integrity and for the normal development and function of the host immune cells (2, 41). IECs secrete thymic stromal lymphopoietin (TSLP), TGF-β and IL-10, which modify the cytokines produced by DCs and macrophages, allowing the expansion and survival of Treg cells with immunosuppressive functions. In addition to TSLP, TGF-β and IL-10, other IEC-derived factors, secretory leukocyte peptidase inhibitor (SLPI), B-cell-activating factor (BAFF), a proliferation-inducing ligand (APRIL) and prostaglandin E_2 (PGE_2), regulate the function of lymphocytes in the intestine (41).

IL-10 is also produced by B- and T-cells, as well as myeloid cells. IL-10 is critical for Treg cell function (55). Treg cells can suppress T-cell-mediated and innate immune pathologies (55-58). Myeloid cell production of IL-10 is important for maintaining forkhead box P3 (Foxp3) expression on Treg cells, which mediates immunosuppressive functions in the colon (59). Polymorphisms found in the IL-10 receptor genes *IL10RA* and *IL10RB* are associated with severe early-onset IBD and polymorphisms in IL-10 itself are also associated with susceptibility to IBD (5,60).

Mice deficient in IL-10 develop spontaneous colitis and colitis-associated cancer, located predominantly in the proximal colon (61). The clinicopathological features of this model resemble CD and are driven by aberrant Th1 cytokine response, dependent on interferon-gamma (IFNγ) and IL-12 (61-63). The disease is exacerbated by *Helicobacter hepaticus* infection and treatment of these mice with IL-12p40 monoclonal antibody ameliorates inflammation (64). Similarly, depletion of local macrophages in the intestine reduces inflammation in *Il10*$^{-/-}$ mice (65). IL-10-secreting macrophages play an important role in immune tolerance and in clearance of apoptotic cells, whereas a deficiency in the latter function may result in exacerbation of inflammation and an increased susceptibility to autoimmune reactions (59, 66). Both epigenetic modifications and pathogenic microflora have been implicated in colitis-associated carcinogenesis in *Il10*$^{-/-}$ mice. Histone deacetylase (HDAC) inhibitors reduce tumour number and size, while treatment of mice with probiotics reduces both inflammation and carcinogenesis in *Il10*$^{-/-}$ mice (67-69). Infection of Il-10-deficient mice with pathogenic bacteria such as *Helicobacter* spp. increases tumour incidence (69). *Paradoxically*, the non-steroidal anti-inflammatory drugs (NSAIDs) celecoxib, rofecoxib and indomethacin exacerbate colitis and increase dysplasia in *Il10*$^{-/-}$ mice, suggesting that in the absence of the immunoregulatory functions of IL-10, NSAIDs contribute to further dysregulation of the immune response (70).

MUC1 is an epithelial cell surface-associated glycoprotein that exhibits altered glycosylation and is overexpressed in the majority of human adenocarcinomas and their precursor lesions (71). Mice transgenic for human *MUC1* (MUC1-Tg) crossed with *Il10*$^{-/-}$ mice demonstrate exacerbation of the colon inflammation, accelerated tumour development compared to *Il10*$^{-/-}$ mice (8-12 weeks), a significant increase of tumours in the proximal colon and profound neutrophilic infiltration (72). These results suggest that aberrant expression of Muc1 in *Il10*$^{-/-}$ mice may promote tumourigenesis by a number of signalling pathways, such as interaction with β-catenin. No mutations of genes frequently involved in CRC have yet been identified in the carcinomas of IL-10-deficient mice (73).

The crucial role of TGF-β in modulating the immune response is now well-established. The main function of TGF-

Table I. *Mouse models of Inflammatory bowel disease (IBD)-associated colorectal cancer.*

Model	Mouse strain	Notes	Timing of cancer	Cancer incidence	Location of tumours	Carcinogenesis modifier factors	Mutations	Ref
IL10$^{-/-}$	C57BL/6J × 129/Ola Severity and AdCa incidence is strain dependent C57BL/6J do not develop AdCas	Presence of activated macrophages and CD4^{+}Th1-like T-cell response, dependent on IFN-γ and IL-12. Goblet cell depletion, multi-focal transmural inflammation, crypt abscesses, mixed inflammatory infiltrate consisting of macrophages, lymphocytes, plasma cells, scattered neutrophils. MHC class II expression on IECs. CD-like phenotype.	3-6 months	60%	Starts at the caecum at 3 weeks and progresses to the proximal and distal colon; can involve the small intestine in aged mice; only the proximal colon is involved in SPF mice.	Decrease in Ca incidence: HDAC inhibitor IL-10 Probiotic bacteria Increase in Ca incidence: *Helicobacter* sp. NSAIDs Human MUC1 transgene	No mutations in *p53*, *K-ras*, *Apc*, no alterations in MSH2 or TGF-β II receptor expression.	(61-64, 67-70, 72, 73)
Rag2$^{-/-}$ × *Tgfβ1*$^{-/-}$	129S6 × CF-1	At 1 week after weaning develop severe hyperplasia, TGF-β-independent inflammation and hyperplasia. TGF-β1 suppresses the transition from hyperplasia to dysplasia.	3-6 months	100%	Caecum and colon	*Helicobacter hepaticus* Mouse strain	No MSI, no alterations in β-catenin expression or *Tgfbr2* mutations detected.	(78, 79)
Smad3$^{-/-}$	129/Sv In 129/Sv × C57BL/6 background reduced penetrance	Hypertrophic, thickened colonic proliferative bowel mucosa, and intense leukocyte infiltrates in the lamina propria. Metastatic AdCa. Mucinous AdCa (with *Helicobacter* spp.)	4.5-6 months ~1-7.5 months (with *Helicobacter* spp.)	100% 50-66%	Proximal and distal colon, caecocolic junction.	*Helicobacter* spp.	APC expression present, increased expression of *c-myc*.	(81, 83)
Rag2$^{-/-}$ with *H. hepaticus*	129/SvEv	Inflammatory infiltrate comprised mainly of macrophages and granulocytes (eosinophils and less frequent neutrophils). Development of invasive and non-invasive carcinoma, small sessile tubular adenomas.	4 months	100%	Common at the caecum and in the colon at 6 months	Treg cells IL-10 Mouse strain-BALB/c and C57Bl/6 less affected IL-6 neutralization TNFα neutralization		(84-86)
TCRb$^{-/-}$ × *p53*$^{-/-}$	C57BL /6JJcl	Inflammatory cell infiltrates in the lamina propria, primarily mononuclear cells. Hyperplasia, dysplasia, AdCa.	4 months	70%	Ileocaecum to the proximal colon	Intestinal microflora required for colitis and colitis-associated cancer	*Apc, p53* mutations, MSI	(87) (97, 98)
IL2$^{-/-}$ × *β$_2$m*$^{-/-}$	C57BL/6 × 129/Ola or 129/Sv mixed background	Mononuclear infiltrate of the mucosa, alterations to the crypt architecture and appearance of crypt abscesses. Clinical features of cancers similar to human UC-associated CRC.	6-12 months	32%	Proximal colon			
Gα$_{i2}$ $^{-/-}$	129/Sv and C57BL/6J	Active chronic colonic inflammation, starts with an increase in lymphocytes and plasma cells in the lamina propria. Infiltration of neutrophils at later stages, crypt loss and depletion of mucus in goblet cells. Crohn's-like lymphocytic infiltrate in the cancer.	4-9 months	31%	75% Proximal including mucinous AdCa in the caecum	Inflammation and cancer development not dependent on specific pathogens.	MSI Inflammation-induced loss of MLH1 and consequently PMS2.	(102, 103)

Table I. *continued*

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Model	Mouse strain	Notes	Timing of cancer	Cancer incidence	Location of tumours	Carcinogenesis modifier factors	Mutations	Ref
SOCS1$^{-/-}$*Tg*		Exogenous SOCS1 is only expressed in T- and B-cells, hyperactivation of STAT1, hyperplasia, loss of goblet cells, crypt abscess formation, and mixed inflammatory infiltrate in the lamina propria after 3 months of age, macrophage infiltration.	6 months	~60% tumour incidence (8 months)	Predominantly proximal colon	IFN-γ prevents tumourigenesis Tumourigenesis not dependent on TNFα	p53 mutations, nuclear β-catenin accumulation.	(104)
Tg(Csf1r-iCre) Jwp$^{+/-}$. *Stat3*$^{flox/flox}$ *Stat3 IKO* (inflammatory cell KO)	C57BL/6	Increased leukocytic infiltration and intestinal mucosal thickening, increased number of macrophages and CD3^{+} cells.	~2-10 months	16%	Caecum and proximal colon	Cancer development dependent on the presence of microbiota.		(105)
Tbx21$^{-/-}$ × *Rag2*$^{-/-}$ (TRUC)	BALB/c background (113) C57BL/6 significantly less severe disease (116)	Mice deficient for T-bet (*Tbx21*) and *Rag2* -(TRUC) develop chronic inflammation resembling UC and colonic dysplasia and rectal adenocarcinoma, COX-2 overexpression in IECs.	>3 months, 6 months significant incidence	42%	Flat AdCA predominantly in the rectum.	Cancer initiation dependent on TNFα, DCs, commensal bacteria.	p53 loss of function, aneuploidy, oxidative DNA damage, β-catenin nuclear localization.	(114, 116)
Gpx1$^{-/-}$/ *Gpx2*$^{-/-}$	C57BL/6J and 129Sv/J or 129S3 mixed background	Acute ileocolitis.	5-12 months	30%	Ileum and colon	Requires bacterial flora, pathogens increase tumour incidence.	β-catenin nuclear accumulation.	(212)
DSS	Swiss Webster Mice, C57Bl/6J	Ulceration, erosion of the epithelial barrier, lymphocyte and granulocyte invasion.	Around 3-7.5 months	25%	Colon, higher incidence in the distal colon.	*p53* *Msh2*	Alterations in β-catenin cell distribution but not in p53.	(122-124, 221, 222)
DSS in *Apc*$^{Min/+}$	C57BL/6J *Apc*$^{Min/+}$	β-Catenin, cyclooxygenase-2, inducible nitric oxide synthase and nitrotyrosine.	4-5 weeks	100%	DSS increases tumour multiplicity and size in the small intestine and induces new colonic tumours.		No *β-catenin* or *K-ras* mutations detected in the AdCa.	(223)
AOM/ DSS	Strain sensitivity Balb/c> C57BL/6N>> C3H/HeN= DBA/2N	DSS is cancer promoter, not inducer, positive nuclear β-catenin, positive for Cox-2 and iNOS. Increased number of mast cells in AdCa.	3-4 weeks	100% 6 weeks	Predominantly distally located.	HDAC inhibitor, *IKKβ* targeted deletion in IECs and myeloid cells, *Il6, Stat3, TNFα, Tlr4, MyD88,* *O*-glycan, Nod1, Inflammasome components	Mutations in the GSK-3β phosphorylation consensus motif of the *β-catenin* gene.	(46, 54, 67, 131, 133, 149, 152, 154, 163-165, 170)

Adenocarcinoma (AdCa), azoxymethane (AOM), adenomatous polyposis coli (Apc), carcinoma (Ca), Crohn's disease (CD), cyclooxygenase 2 (Cox-2), colorectal cancer (CRC), dendritic cells (DCs), glutathione peroxidase 1/2 (Gpx1/2), glycogen synthase kinase 3 beta (GSK-3β), histone deacetylase (HDAC), interferon-gamma (IFN-γ), intestinal epithelial cells (IECs), v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (K-ras), major histocompatibility complex (MHC), mutL homolog 1 (MLH1), mutS homolog 2 (MSH2), microsatellite instability (MSI), myeloid differentiation primary response gene (88) (MyD88), nucleotide-binding oligomerization domain containing 1 (NOD1), non-steroidal anti-inflammatory drugs (NSAIDs), PMS2 post-meiotic segregation increased 2 (S. cerevisiae) (PMS2), recombination activating gene 2 (Rag2), suppressor of cytokine signaling 1 (SOCS1), specific pathogen-free (SPF), signal transducer and activator of transcription 1/3 (STAT1/3), transforming growth factor beta (TGF-β), transforming growth factor beta receptor 2 (Tgfbr2), Toll-like receptor 4 (TLR4), tumour necrosis factor alpha (TNFα), ulcerative colitis (UC), β_2-microglobulin (β_2m).

β is to maintain T-cell tolerance to self or harmless antigens through its regulatory effects on effector and regulatory T-cells (74). T-Cells from patients with IBD showed unresponsiveness to TGF-β through up-regulation of SMAD family member 7 (SMAD7), a negative regulator of TGF-β signalling (75). TGF-β receptor type II (*TGFBR2*) gene is mutated through mismatch repair deficiency in a small subset (6%) of UC-associated neoplasms (76). TGF-β1 deficiency in mice results in multiorgan inflammation and early death (77). When crossed with immunodeficient recombination activating gene 2 $(Rag2)^{-/-}$ mice, the double-knockout mice survive longer and develop inflammation-associated colonic hyperplasia and carcinoma. The model establishes the protective role of TGF-β1 in early cancer promotion in immunocompromised animals by maintaining epithelial tissue organization and suppressing the transformation from hyperplasia to dysplasia (78). Carcinogenesis in this model is dependent on infection with pathogens and the genetic background of the mouse (79). TGF-β signalling is mediated by the SMAD family of intracellular proteins, including SMAD2, SMAD3 and SMAD4 (80). $Smad3^{ex2/ex2}$-deficient mice also develop chronic intestinal inflammation and frequent caecal and colonic adenocarcinomas (81). However, in another report, $Smad3^{ex8/ex8}$-deficient mice showed infrequent colon adenomas at the age of 6 months (82). This discrepancy may be due to the different pathogen status of the animals in the two studies. *Helicobacter*-infected but not *Helicobacter*-free $Smad3^{-/-}$ mice have been found to develop colon cancer (83).

Mice with Defects in the Adaptive Immunity

$Rag2^{-/-}$ mice are characterised by a lack of mature B- and T-cells. When infected with *H. hepaticus* these mice develop colon inflammation, hyperplasia, dysplasia and carcinoma. Both inflammation and malignant changes can be reduced by transfer of Treg cells (characterized by $CD4^+CD45RB^{lo}CD25^+$ expression) but not by Treg cells from IL-10-deficient donors. This establishes the crucial role of IL-10 competent Treg cells in the restoration of epithelial integrity and prevention of pathogen-induced inflammation and cancer (84-86). In addition, neutralization of IL-6 reversed the invasive mucinous adenocarcinoma phenotype in this model. Mice receiving IL-10–Ig fusion protein exhibited down-regulation of IL-6 in response to *H. hepaticus* infection (86). Thus, the study establishes a model in which IL-10 suppresses pathogen-induced elevation of IL-6 and carcinogenesis.

Mice deficient in T-cell receptor β chain lack mature T-cells and develop colitis resembling UC. When crossed with *p53*-deficient mice, the double-knockout mice develop a high frequency of adenocarcinoma. Both the inflammatory response and cancer development are dependent on the intestinal microflora as these mice do not develop tumours in microbe-free conditions (87).

IL-2 is an important regulatory cytokine for T-cell growth and expansion and plays a central role in the differentiation of naive $CD4^+$ T-cells into Th2 cells (88). Furthermore IL-2 is essential for Treg cell growth and for sustaining the Treg cell population in order to mediate immune homeostasis and tolerance to self (89). Young (3 to 4-week-old) IL-2-deficient mice have normal immune responses with no apparent intestinal or hepatic inflammation; however, older mice develop colitis mediated by $CD4^+$ T-cells (90-92). The initiation of the disease is dependent on thymus-derived T-cells that infiltrate the colon and bone marrow, inducing colitis, lymphoadenopathy, splenomegaly, loss of B-cells and anemia (93). Strikingly, microbe-free $Il2^{-/-}$ mice do not develop colitis in contrast to specific pathogen-free (SPF) $Il2^{-/-}$ mice, while the extraintestinal pathology is independent of the microbial flora. However microbe-free $Il2^{-/-}$ mice exhibit abnormalities in the generation and maintenance of T-cell receptor gamma delta (TCRγδ+) subsets of intestinal intraepithelial lymphocytes (94), which are known to accumulate in the inflamed tissue in CD and UC (95). Despite the severe colitis, 6-month-old $Il2^{-/-}$ mice do not develop gastrointestinal cancer. However, mice deficient in both IL-2 and β_2-microglobulin (a component of MHC class I molecules) develop colitis with faster onset compared to $Il2^{-/-}$ mice (96). These mice lack $CD8^+$ T-cells and live longer due to less pronounced systemic disease. By 6 months of age they develop proximally located adenocarcinoma, characterized by mutations in the *Apc* and *p53* genes, and MSI (97, 98). Interestingly, mutations in the β_2-microglobulin gene have been detected in colorectal tumours and therefore these cells have reduced MHC class I expression and are not recognized by $CD8^+$ T-cells. An increased density of $CD8^+$ cytotoxic lymphocytes in colonic tumours is associated with a good prognosis, suggesting that an escape of immune recognition by cytotoxic $CD8^+$ T-cells may be a mechanism for tumour progression in inflammation-associated CRC (99, 100).

Mice with Defects in Signal Transduction and Transcription Factors

G-Proteins are heterotrimeric guanine nucleotide binding proteins that regulate signal transduction through adenylyl cyclases, phospholipase C and ion channels (101). Mice deficient in the G-protein subunit α_{i2} have defects in T-cell maturation and function, such as defective chemotactic migration of thymic and colonic T-cells, and UC-like inflammation with mucinous adenocarcinoma predominantly located in the proximal colon (102). These tumours exhibit MSI and epigenetic silencing of the *Mlh1* promoter that is mediated by the transcriptional repressor DEC-1 and is dependent on inflammatory hypoxia (103).

Suppressor of cytokine signalling-1 (SOCS1) is an intracellular protein that inhibits janus kinase (JAK)-mediated cytokine signalling. SOCS-1 deficient mice die

shortly after birth but mice with restoration of SOCS1 specifically in T- and B- cells (SOCS1$^{-/-}$Tg) survive for more than one year. These mice develop spontaneous colitis at 3 months of age and cancer by 6 months of age, characterized by IFNγ up-regulation and hyperactivation of signal transducer and activator of transcription 1 (STAT1) signalling. Constitutive activation of STAT3 and NF-κB signalling pathways were also observed in SOCS1$^{-/-}$Tg mice, but their activity was not a contributing factor to tumourigenesis (104). In contrast, inactivation of IFNγ suppressed STAT1 hyperactivation and tumourigenesis in this model, implicating SOCS1 activity as a tumour suppressor and an important regulator of IFNγ signalling (104).

STAT3 inactivation specifically in haematopoietic cells (mainly in macrophages, with partial deletion in other myeloid and lymphoid cells) results in colitis and inflammation-associated colonic cancer in mice (105). Since STAT3 functions as a major mediator of IL-10 signalling and IL-10 has potent immune suppression functions, it is possible that chronic inflammation in this model is induced due to unregulated activation of both myeloid and lymphoid cells. Microbiota are required for both the development of inflammation and tumourigenesis in this model. The chronic inflammatory environment leads to disruption of the epithelial barrier and to epithelial hyperproliferation, associated with up-regulation of mammalian target of rapamycin (mTOR) and STAT3 activity in colonic epithelial cells. Furthermore, epithelial hyperproliferation was mTOR-dependent and aberrant upregulation of mTOR-STAT3 crosstalk was observed in the mucosa of patients with IBD (105).

In summary, immunoregulatory functions of T-cells mediated by IL-10 and TGF-β signalling contribute to mucosal homeostasis and prevent abnormal immune responses to the intestinal microflora. In addition, disruption of signal transduction pathways regulating T-cell maturation and function, as well as uncontrolled cytokine signalling, contribute to the onset of colitis. The sustained inflammatory response creates a landscape associated with an increase in pro-oncogenic cytokines, such as IL-6, and hypoxic conditions that may promote silencing of DNA repair genes and induce genetic instability (86, 103). The introduction of additional defects such as p53 and β_2-microglobulin deficiency accelerate tumourigenesis in the context of defective adaptive immunity and chronic inflammation (87, 97, 98).

The Role of DCs in Inflammation and Inflammation-associated Colorectal Carcinogenesis

DCs act as 'sentinels' for the presence of pathogenic bacteria and play a key role in regulating immune homeostasis and tolerance in the colon. DCs can be seen as a regulatory hub that integrates different environmental signals and subsequently 'issues' appropriate directions to the adaptive immunity, thereby shaping the immune response. Therefore aberrant DC function may directly contribute to the pathogenesis of IBD and IBD-associated cancer (106). DCs extend dendrites into the colonic lumen to sample and evaluate commensal and pathogenic bacteria (107-109). DCs also receive signals from the IECs, which secrete factors such as TSLP and retinoic acid that 'condition' DCs to promote the development of Th2 cells and Treg cell subsets with tolerogenic functions (110, 111).

T-bet (encoded by the *Tbx21* gene) is a T-box transcription factor family member that regulates type 1 inflammatory immune response in both adaptive and innate immunity (112). T-bet is required for the optimal production of IFNγ by DCs (113). Mice deficient in Rag2 and T-bet transcription factor [*T-bet*$^{-/-}$ x *Rag2*$^{-/-}$ UC (TRUC) mice] develop inflammation resembling UC and colonic adenocarcinoma (114, 115). Malignant changes in TRUC mice are driven by T-bet deficiency in DCs and exhibit molecular alterations that are characteristic of cancer in patients with UC, such as p53 loss of function, overexpression of cyclooxygenase 2 (Cox-2) and aberrant expression of β-catenin. Carcinogenesis is driven by dysregulated expression of TNFα and is dependent on commensal bacteria. Targeted re-expression of T-bet in DCs ameliorated colitis and carcinogenesis and reduced the levels of TNFα, suggesting that T-bet may act as a repressor of TNFα in DCs. The severity of the colitis, as well as dysplasia and adenocarcinoma, in the TRUC model is dependent on the murine strain background and is controlled by the cytokine deficiency-induced colitis susceptibility-1 (Cdcs1) locus on chromosome 3 through the innate immune cells (116). This highlights the importance of T-bet dysfunction in DCs and strain-specific genetic modifiers in the promotion of inflammation-associated cancer.

Mouse Models with Chemically-induced Colitis and Cancer

DSS model. Chronic inflammation resembling UC can be induced by oral administration of DSS, which triggers inflammation by damaging the gut-epithelial barrier (53, 117). In the DSS model, inflammation is accompanied by generation of reactive oxygen species (ROS) and a decrease in the antioxidant defense of the inflamed mucosa (118, 119) similar to IBD (120, 121). Repeated cycles of DSS are required to induce carcinogenesis in a subset of mice. However, genetic defects, such as mutations in key tumour suppressor genes, accelerate tumour promotion in the context of DSS-induced inflammation. For example, 60% of mice deficient for the DNA mismatch repair gene *Msh2* developed colonic dysplasia or adenocarcinoma with the DSS treatment, compared to 29% of their wild type siblings. Untreated *Msh2*$^{-/-}$ mice develop spontaneous tumours in the small intestine but not in the colon (122). Thus, defects in the DNA mismatch repair genes

accelerate tumourigenesis in the context of chronic inflammation and can shift the gastrointestinal location of tumour formation. Although repeated cycles of DSS induce proximal and distal colon inflammation, the inflammatory response is more severe in the distal colon and this is associated with the majority of cases of dysplasia and cancer (123). p53 deficiency also increases the number of flat cancer lesions in the DSS model (124).

Azoxymethane (AOM)/DSS model of colitis-associated cancer. Inflammation-associated cancer induced by DSS in mice requires prolonged time and exposure to repeated cycles of DSS. Injection of the carcinogen AOM prior to exposure to DSS reduces the time required for cancer development (125). The AOM/DSS model of inflammation-associated cancer has been widely utilized to study the molecular factors that trigger malignant transformation and promote tumour growth. This model is frequently referred to as colitis-associated cancer model (CAC).

Insights into the Mechanism of Carcinogenesis from the CAC Model

Pro-tumorigeneic role of NF-κB. NF-κB, a family of transcription factors, is a master regulator of gene expression with key roles in orchestrating pro- and anti-inflammatory responses, as well as cell survival and differentiation programs. Increase in NF-κB activity has been found in human colorectal cancer samples and in colon cancer cell lines (126, 127). In patients with Crohn's disease, there is an increase in NF-κB in epithelial cells as well as in infiltrating macrophages (128), indicating that NF-κB activity is involved in colon inflammatory pathogenesis. Similarly, NF-κB activity is up-regulated in the *Il10*$^{-/-}$ mouse model of chronic colon inflammation, and administration of anti-sense oligonucleotide to the RelA subunit of NF-κB alleviates the symptoms of the disease (129). NF-κB target genes include cytokines and chemokines, which participate in tumour promotion in the context of chronic inflammation. NF-κB is involved in the up-regulation of genes that are associated with tumourigenesis such as *TNFα, IL-1β* and *IL-8* (15). NF-κB also controls the expression of anti-apoptotic genes such as B-cell lymphoma 2 (*Bcl-2*) and B-cell lymphoma-extra large (*Bcl-xL*) (130). One of the most convincing pieces of evidence that NF-κB activity is implicated in cancer comes from studies of the CAC model. Enterocyte-specific ablation of IKKβ, an essential kinase in NF-κB signalling, resulted in a decrease in colitis-associated tumour incidence (131). In this context, epithelial NF-κB functions as a pro-tumourigenic factor due to induction of anti-apoptotic genes such as *Bcl-xL*.

NF-κB signalling may have different or even opposing roles depending on the tissue context or grade of inflammation. NF-κB loss in epithelial cells during acute DSS-induced inflammation hinders mucosal healing and prevents recruitment of inflammatory cells that produce cytoprotective factors such as IL-11 and IL-22. In contrast, inactivation of epithelial NF-κB signalling has no effect in the *Il10*$^{-/-}$ mouse model of chronic inflammation but NF-κB ablation in myeloid cells ameliorates inflammation in the same model (132). NF-κB signalling promotes cancer in the AOM/DSS model but through different mechanisms in the colonic epithelial and myeloid cell components. Conditional ablation of IKKβ in enterocytes reduces tumour incidence by 80% in this model, possibly by increasing apoptosis of premalignant cells, whereas deletion of IKKβ in myeloid cells (macrophages, neutrophils, DCs) results in a significant reduction of tumour size. These elegant studies demonstrate that NF-κB signalling in myeloid cells results in up-regulation of pro-inflammatory factors that function as tumour growth factors (131). One of the cytokines implicated in this process is IL-6, and the IL-6–STAT3 signalling axis has been shown to regulate proliferation and survival of tumour initiating IECs (133).

IL-6, IL-21 and TNFα are major tumour-promoting cytokines. IL-6 was identified as a critical NF-κB-dependent cytokine that is produced by myeloid cells of the lamina propria (mainly DCs and macrophages). IL-6 stimulates survival and proliferation of IECs through STAT3 activation downstream of the gp130 receptor and promoted tumourigenesis in the AOM/DSS model (133). This study identified the importance of trans-signalling cross-talk between the immune compartment and the IECs in response to acute colitis and the critical role of myeloid-produced factors in tumour promotion. IL-6 and pSTAT3 overexpression have been detected in the epithelial cells of patients with active UC (134). Similarly, mice with IEC-specific deficiency in SOCS3, a negative regulator of receptor mediated activation of STAT3, exhibit increased tumourigenesis in the AOM/DSS model (135). IL-6 also plays an important role in intestinal homeostasis and epithelial regeneration, and can activate three signalling cascades: SH2-containing phosphotyrosine phosphatase (SHP2)–Ras–extracellular signal-regulated kinase (ERK), JAK1/2–STAT3 and phosphoinositide-3-kinase (PI3K)–v-akt murine thymoma viral oncogene homolog 1 (AKT)–mTOR (136-138). However, IL-6 can further propagate inflammatory signals through the regulation of pathogenic T-cells. IL-6 inhibits the generation of the anti-inflammatory Foxp3^{+} Treg cells and instead, in concert with TGF-β, promotes generation of pathogenic Th17 from naïve T-cells (139). Recently it has been demonstrated that in addition to driving gp130-mediated STAT3 activation, IL-6 drives the overexpression of vascular endothelial growth factor receptor 2 (VEGFR2) in IECs in the CAC model, thereby enhancing STAT3 activation through VEGF signalling, which further promotes epithelial cell proliferation and tumour growth (140).

IL-21 is expressed by $CD4^+$ cells, including Th1 and Th17 cells, and has been shown to promote Th17 responses (141). IL-21 is overexpressed in the mucosa and tumours of patients with UC (142). IL-21 deficiency in AOM/DSS-treated mice results in a reduction in tumour load, paralleled by a decrease in IL-6 and IL-17A expression (142). IL-21 is a negative regulator of $CD4^+Foxp3^+$ Treg cells and these cells are increased in the colon of IL-21-deficient mice, providing yet another affirmation of the cancer suppressor role of Treg cells. As IL-21 is produced by $CD4^+$ T-cells and IL-21R is broadly expressed by T- and myeloid cells, it is likely that IL-21 promotes tumour growth through paracrine/autocrine mechanisms inducing the production of the pro-carcinogenic IL-6 (predominantly produced by myeloid cells) and IL-17A (produced by T-cells).

Although TNFα was initially associated with tumour necrosis, mounting evidence suggested that TNFα plays a role in tumour growth and progression (143). TNFα signalling contributes to mutagenic ROS generation, and induction of metalloproteases and genes involved in inflammation, tissue repair and angiogenesis as well as recruitment of activated leukocytes to the site of inflammation (144-148). Anti-TNFα therapy is successful in a subset of patients with IBD (2). Suppression of TNFα signalling in the mouse model of CAC reduces tumourigenesis, inflammation and infiltration of macrophages and neutrophils (149). Similarly, TNFα neutralization prevents cancer formation in other mouse models of inflammation-associated cancer.

Dysregulation of pattern recognition receptor signalling promotes tumourigenesis. Pattern recognition receptors (PRRs) sense microbial agents and play a crucial role in the mucosal homeostasis. Aberrant activation of PRRs is a major contributor to IBD in humans and in mouse models. The innate immune system has several classes of PRRs. Toll-like receptors (TLRs) sense microbes on the cell surface and endosomes, while nucleotide-binding oligomerization domain containing (NOD)-like receptors (NLRs), such as NOD1 and NOD2, and retinoid-inducible gene 1 (RIG-1)-like receptors (RLRs) recognize cytosolic microbial components.

Signalling through these receptors triggers several downstream kinases, some of which are involved in up-regulation of cytokines and chemokines (150,151). TLR signalling activates NF-κB and AP-1 transcription factors through the TLR adaptor MyD88. However, mice deficient in MyD88 exhibit susceptibility to DSS-induced colitis and an increased mortality due to defects in epithelial regeneration and tissue repair (36). MyD88-deficient mice also demonstrate dramatically increased tumourigenesis in the CAC model (152). Remarkably, these mice do not demonstrate increased epithelial cell proliferation observed in other models of inflammation-associated cancer, but instead, have alterations in the inflammatory microenvironment associated with an increased expression in wound healing factors such as IL-6, IL-11, EGFR ligands, COX-2 and hypoxia-inducible factor 1 alpha (HIF-1α) (152). These mice also express phosphorylated STAT3 in the nuclei of IECs, suggesting activation of the pro-tumorigenic IL-6/IL-11–STAT3 cascade (136).

Dysregulated epithelial cell–microbiota interaction through uncontrolled TLR signalling can also contribute to colitis. Mice deficient in the single immunoglobulin IL-1 receptor-related molecule (SIGIRR), a negative regulator for Toll–IL-1R signalling, exhibit commensal bacteria-dependent defects in epithelial cell homeostasis, constitutive expression of pro-inflammatory mediators, hyperactivation of NF-κB and STAT3, susceptibility to DSS-induced colitis and increased AOM/DSS-induced tumourigenesis (153).

TLR4 is overexpressed in the malignant tissues of patients with UC as well as in AOM/DSS-induced cancer in mice (154). Unlike *MyD88*$^{-/-}$ mice, TLR4-deficient mice are protected from AOM/DSS-induced tumourigenesis. The acute phase of inflammation is ameliorated in TLR4-deficient mice and this is associated with a decrease in NF-κB signalling, as well as a down-regulation of the pro-tumorigenic COX2/PGE_2. PGE_2, produced downstream of TLR4 activation, can act in a paracrine or autocrine manner to stimulate the expression and secretion of the epidermal growth factor receptor (EGFR) ligand amphiregulin. Amphiregulin activation of EGFR results in an increased proliferation of colonocytes that may contribute to oncogenic transformation (154). PGE_2 deficiency confers significant protection against AOM-induced colonic tumours (155). Remarkably, loss of PGE_2 reduces the expansion of $CD4^+Foxp3^+$ Treg cells with immunosuppressive functions in the colon draining mesenteric lymph nodes in mice, unveiling a potential mechanism of how loss of PGE_2 modifies the tumour inflammatory microenvironment and promotes antitumour immunity (155).

The inflammasomes and IL-18 activity suppress CAC. A set of NLRs result in the activation of caspase-1 through an assembly of large protein complexes termed inflammasomes (156). Caspase-1 activity has a critical function in the secretion and maturation of IL-1β and IL-18 (157, 158). Inflammasomes are categorized into subgroups according to the major components NLRP1, NLRP3, NLRC4, NLRP6 and absent in melanoma 2 (AIM2) (159). The role of inflammasomes and NLR in inflammatory disease has currently taken central stage (2). Genome-wide association studies (GWAS) have identified IBD-associated polymorphisms on *NLRP3* and *IL18RAP* (160, 161). Levels of IL-18 and IL-1β are increased in patients with IBD (162).

Oncosuppressive functions have been attributed to inflammasomes. Inflammasomes can eliminate malignant cells through induction of apoptosis (159). For example, *Nlrc4*$^{-/-}$ and *Casp1*$^{-/-}$-deficient mice exhibit resistance to apoptosis and increased epithelial cell proliferation in response to injury

(163). Similarly, deficiency of NLRP6 in mouse IECs results in reduction of IL-18 levels, accompanied by quantitative and qualitative changes in numerous bacterial taxa (dysbiosis). The dysbiosis in NLRP6 inflammasome-deficient mice mediates transmissible autoinflammation, spontaneous hyperplasia and exacerbation of DSS-induced colitis (164).

An antitumour function has been assigned to NLRP3. Mice deficient in *Casp1* and *Nlrp3* exhibit increased susceptibility to DSS-induced colitis and reduced tumour load due to down-regulation of IL-18 expression. The role of IL-18, in this context, appears paradoxical because IL-18 is important for maintenance of the epithelial integrity and proliferation in response to injury in the acute phase of inflammation. However, in the chronic inflammatory environment, IL-18 acts as a tumour suppressor by inhibiting epithelial cell proliferation, a function that may be mediated in part by IFNγ antitumor activities through STAT1-dependent signalling (165, 166). Furthermore, IL-18 promotes IFNγ production in activated T- and natural killer (NK) cells, thus promoting Th1 cell polarization that may contribute to its antitumourigenic role (167, 168). In contrast to NLRC4, which exerts its antitumorigenic activity through its effects on IECs, NLRP3 suppresses tumourigenesis through its activity in the haematopoietic/myeloid compartment (169). Deficiency in another PRR involved in maintaining the epithelial integrity, NOD1, results in an increased susceptibility to DSS-induced acute injury associated with cytokine production and an increase in epithelial cell proliferation. *Nod1*$^{-/-}$ mice exhibit increased colitis-associated tumourigenesis where tumour development is dependent on the presence of intestinal microbiota (170).

Is HIF an Unappreciated Target of Inflammation-associated CRC?

The gastrointestinal tract functions in a state of low-grade inflammation and in rapid, drastic changes in tissue oxygen availability (171). Hypoxia-inducible factor (HIF) plays a central role in maintaining the epithelial barrier and in promoting cell survival under hypoxic conditions [reviewed in (171)]. HIF is rapidly degraded under normoxia and is stabilized in hypoxia. HIF is a heterodimeric transcription factor and consists of a constitutively expressed HIF-1β subunit and a highly regulated HIF-1α, or the related HIF-2α and HIF-3α subunits (172). HIF regulates the expression of genes mainly involved in adaptation to hypoxic microenvironments, such as genes coding enzymes from the anaerobic glycolysis pathway and genes implicated in angiogenesis. HIF-1 expression has been documented in cells of the innate and adaptive immune system, as immune cell function necessitates rapid adaptation to varying oxygen tissue tension. Highly proliferating cells, such as cells of the immune system, use glycolysis as their primary energy production pathway (173-176). As most of the enzymes of the glycolytic pathway are target genes of HIF-1, HIF-1 activity is essential for the function of the immune system.

HIF-1α appears to have different functions and importance in different cell types of the immune system. HIF-1α protects from activation-induced cell death in peripheral T-cells under hypoxic conditions (177). However, HIF-1 activation in thymocytes leads to caspase-8-mediated apoptosis (178). HIF-1α deficiency is embryonically lethal but was bypassed by the generation of Hif1α$^{-/-}$ → Rag2$^{-/-}$ chimeric mice. In these mice, HIF-1α deficiency in B- and T-cells leads to an autoimmune reaction and abnormal B-lymphocyte development (179). HIF-1α in myeloid cells is essential for their invasiveness, motility, aggregation and bacterial killing (176). A range of cytokines with pro-tumourigenic role can affect HIF-1 regulation. Some of them include TGF-β1, TNFα, IL-1β (180, 181). Conversely, HIF-1α can also modulate the expression of cytokines, such as IL-1, IL-4, IL-6 and IL-12 in macrophages (182). In addition, there is substantial evidence for a bi-directional relationship between HIF-1 and NF-κB, each enhancing the activity of the other (183,184).

Apart from inflammatory cells, HIF is also expressed in epithelial cells. HIF overexpression in colonic epithelial cells increased inflammatory infiltration and induced colonic oedema under normal conditions and resulted in exacerbation of DSS-induced colitis (185). Therefore, aberrant HIF-1 activation in epithelial cells may disturb the precise regulation of the inflammatory response and result in exacerbation of pathological conditions. Different strategies to inhibit HIF-1 signalling have been proposed for the treatment of inflammatory disorders (186). On the other hand, an increase in HIF-1 through inhibition or deficiency of prolyl hydrohylases (PHDs), the main hydroxylases promoting HIF-1 degradation, has been found to be protective in murine models of colitis (187-189). Therefore HIF-1-related strategies have been proposed for the treatment of chronic intestinal disorders. These strategies aim to stabilize HIF-1 expression and HIF-mediated maintenance of epithelial barrier function and induction of barrier protective factors (186, 190-192). However, PHD inhibitors are not specific to HIF-1 protein level stabilization. PHD inhibition also leads to an increase in NF-κB activity (193). Therefore, it is unknown whether the protective effect of PHD can be attributed mainly to increased levels of HIF-1 or to an increase in NF-κB activity.

HIF-1α overexpression has been observed in numerous types of cancer, including colon, breast, gastric, lung, skin, ovarian, pancreatic, prostate and renal tumours (194). However HIF-1 has not been well studied in relation to colon inflammation-induced oncogenesis. Recently, overexpression of microRNA-31 (miR-31) was found in IBD-related neoplasia. miR-31 increases HIF-1α expression through targeting its inhibitor factor, inhibiting HIF-1 (FIH-1). In this study miR-31 expression was a unique feature of IBD-

associated cancer and was not found in sporadic CRC. Furthermore, miR-31 increased significantly during the transformation from normal epithelium to dysplasia and from dysplasia to cancer (195). Thus, this study suggests a role for HIF-1 in the malignant transformation in the context of IBD.

Polymorphisms in genes involved in the Th17 signalling pathway are associated with IBD (2, 196). Th17 cells are key players in the anti-inflammatory response to pathogens in the intestine, however, Th17 signalling can also drive autoimmune disorders, IBD, and inflammation-associated colon cancer (197, 198). HIF-1α was recently found to play a pivotal role in the development of Th17 cells and in the regulation of the Th17:Treg cell balance (199). Thus HIF-1 expression may participate in a vicious circle of propagating inflammation through promoting Th17 differentiation and IL-17 signalling, which in turn can promote sustained HIF-1 action (199).

HIF-1α overexpression in IECs was found in a mouse model of NSAID-induced inflammation-associated colon cancer (200). Although the tumour-promoting role of HIF-1α in this model requires further study, IEC-specific ablation of HIF-1α ameliorated the inflammatory response induced by the NSAID sulindac. In this model, oral administration of sulindac induced inflammatory lesions with serrated neoplasia which were most pronounced on the mucosal folds of the proximal colon. The lesions displayed mild to moderate acute and chronic inflammation, progressing to serrated neoplasia and mucinous adenocarcinoma in genetically-modified mice. In an experiment comparing *Msh2* and *p53*-deficient mice with their wild-type siblings, adenocarcinoma was observed in up to 25% of the knockout mice on the C57Bl6J background. The wild-type siblings also developed inflammatory lesions on the sulindac diet but adenocarcinoma was rare. Sulindac-treated mice in this experiment had only a few lesions intercepted by macroscopically normal appearing mucosa, and few or no external symptoms of IBD. Microscopic analysis revealed areas of early surface erosion and rare ulceration in the non-neoplastic mucosa, suggesting that damage to the mucosal barrier may play a role in carcinogenesis in this model (200).

Both malignant and premalignant lesions were characterized by a marked overexpression of the pro-tumourigenic factors MIP-2 (the murine IL-8 homologue), IL-1β and COX-2, as well as HIF-1α (200). IL-8 plays a key role in cancer by initiation of tumour-associated inflammation, angiogenesis, proliferation and survival of endothelial and cancer cells (201, 202). Remarkably, in the distal colon of mice in the same model (200), sulindac prevented AOM-induced tumours, consistent with its role as a chemopreventive agent. This model may be useful for the study of proximal colon carcinogenesis and the serrated neoplasia pathway, which is characterized by proximal location and HIF-1α expression (203). Further analysis is required to determine how the sulindac model (200) compares with other mouse models of inflammation-associated colon cancer discussed in this review.

The Role of the Microbiota in Inflammation-associated CRC

While the relationship of microbiota with colitis and inflammation-associated CRC is one of the most rapidly expanding fields, it is not the focus of this review, however, a few points will be made here.

One of the most interesting recent advances in our understanding of the gut-colonizing bacteria is that their functions extend far beyond aiding digestion. The intestinal bacteria actively shape and influence not only the gut epithelial and immune homeostasis but also extraintestinal systems such as the cardiovascular and nervous systems (204-206). A 'Western-style' diet alters the microbiotic composition and induces adiposity in humanized gnotobiotic mice (adult human fecal microbial communities transplanted into microbe-free mice) and this trait is transmissible to non-obese mice through microbiotic transplantation (207). Diet is not the only factor that controls bacterial populations. Defects in innate immunity have a marked influence on microbiotic composition and the shift of microbial communities can induce communicable (transmissible) pathologies, such as colitis and metabolic syndrome in mice (208, 209). A UC twin study recently found a distinct microbial species composition and decreased diversity in the mucosa of patients with UC compared to their twin healthy siblings. The presence of certain bacterial genera correlated with host mucosal gene expression, indicating the interdependency of microbiotic metabolism and the host transcriptome (210).

How do microbiota contribute to inflammation-associated cancer? As discussed in this review, a large proportion of mouse IBD-related carcinomas require interaction with commensal or pathogenic bacterial species, suggesting that colitis is required but is not sufficient to induce cancer in some models (79, 197, 211, 212). The main mechanisms of bacterial-induced carcinogenesis are either through microbiota-mediated uncontrolled pro-inflammatory signalling or through exerting cytotoxic effects on mucosal cells (direct or mediated through microbial metabolites/activated host cells) (204). For example, the best known cancer-causing pathogen *H. pylori* has been proposed to induce carcinogenesis through persistent T-cell-mediated immune response and production of DNA-damaging ROS (204, 213-215). Other bacterial species have different mechanisms of promoting CRC (15). Mice deficient in the antioxidant enzymes glutathione peroxidase 1 and 2 develop microflora-associated intestinal inflammation and tumourigenesis, establishing the crucial role of antioxidant defense against bacteria-induced oxidative stress and cancer (212).

The microbiotic composition has a spatial variation along the axis of the large intestine (216). Despite the fact that the intestinal epithelial barrier is separated from the microflora by a thick mucus layer, bacteria interact directly with the intestinal crypts and significantly more crypts of the proximal colon are in contact with bacteria compared to crypts in the middle and distal colon (216). In *Smad3*$^{-/-}$ mice, *Helicobacter bacteria* preferentially colonized the caecum, and to a less extent, the proximal colon compared to the distal colon, reflecting the predominant site of tumourigenesis in these mice (83).

Perspective and Conclusion

Based on animal models, the traditionally proposed mechanisms of cancer induction by chronic inflammation include tissue damage and subsequent regenerative hyperproliferation of epithelial cells. The chronic inflammatory microenvironment is rich in macrophages that participate in the generation of ROS (217). ROS are mutagenic agents and combined with increased cellular proliferation at the site of inflammation, ROS can trigger oncogenic transformation (217, 218). As the inflammatory microenvironment is rich in both mutagenic ROS and secreted cytokines and growth factors, which can stimulate cell proliferation, cells with DNA damage may be allowed to go through the cell cycle before efficient DNA repair has occurred. Thus, potentially oncogenic mutations can be transmitted to progeny cells. In chemically-induced liver carcinogenesis, for example, compensatory proliferation causes initiated hepatocytes to enter the cell cycle and pass mutations onto the daughter cells (219, 220). However, mutations in classic tumour suppressor genes are not always found in inflammation-associated colon cancer in the mouse. For example, in the IL-10-deficient mouse model of colitis-associated cancer, no mutations in *p53, K-ras* and *Apc* have been found yet, indicating that IBD-related cancer may exploit additional/alternative oncogenic pathways or epigenetic mechanisms (15, 62, 73).

The increased rate of epithelial proliferation in response to mucosal barrier damage may accelerate the normal age-related epigenetic changes occurring in the colon mucosa. This in turn may leads to silencing of key tumour suppressor or DNA damage repair genes (67, 103). One of the key features of most mouse models of inflammation-associated CRC is that they require prolonged exposure to the inflammatory microenvironment, the introduction of additional an genetic defects 'precipitates' cancer initiation. These additional defects can be caused by treatment with carcinogens or by using mice with tumour suppressor gene mutations. Therefore chronic inflammation by itself may not be sufficient to induce oncogenic transformation. Even in the same model, some mouse strains are more susceptible to inflammation-associated cancers than others, implying that genetic variation also plays a key role. Another important point is that the inflammatory response affects the whole colon in many mouse models of colitis, however, cancer develops preferentially in the caecum or the proximal and distal ends of the colon. As discussed above, this may be due to regional differences in bacterial colonisation or perhaps intrinsic molecular differences of colonocytes or specialised mucosal immunity in different colonic segments. A pitfall of the introduced animal models is that they rarely involve the ileum, which is usually affected in CD (197). It can be speculated that disturbances in mucosal homeostasis regardless of the initiating factor lead to dysregulated, sustained and hyperactivated host inflammatory response to microbiota and drive carcinogenesis. Alternatively it can be proposed that the prolonged host immune dysregulation re-structures microbial communities in the gut, allowing the thriving of pathogenic species, which promote carcinogenesis.

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