The inflammatory pathogenesis of colorectal cancer

Mark Schmitt[1,2] *and Florian R. Greten*[1,2,3]✉

Abstract | The mutational landscape of colorectal cancer (CRC) does not enable predictions to be made about the survival of patients or their response to therapy. Instead, studying the polarization and activation profiles of immune cells and stromal cells in the tumour microenvironment has been shown to be more informative, thus making CRC a prototypical example of the importance of an inflammatory microenvironment for tumorigenesis. Here, we review our current understanding of how colon cancer cells interact with their microenvironment, comprised of immune cells, stromal cells and the intestinal microbiome, to suppress or escape immune responses and how inflammatory processes shape the immune pathogenesis of CRC.

Stage 4 colorectal cancer
The stage at which the colorectal cancer has spread to other parts of the body such as lung, liver, abdominal wall, ovary or distant lymph nodes.

Aneuploidy
The presence of an abnormal number of chromosomes in a cell.

Loss of heterozygosity
Describes a cross-chromosomal event that results in loss of the entire gene and the surrounding chromosomal region.

Microsatellite instability
(MSI). The phenotypic indication of non-functional DNA mismatch repair that results in genetic hypermutability.

[1]*Institute for Tumor Biology and Experimental Therapy, Georg-Speyer-Haus, Frankfurt/Main, Germany.*

[2]*Frankfurt Cancer Institute, Goethe University Frankfurt, Frankfurt/Main, Germany.*

[3]*German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg, Germany.*

✉*e-mail: greten@gsh.uni-frankfurt.de*

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Colorectal cancer (CRC) is the third most prevalent cancer worldwide. Despite certain improvements in screening and therapy, the incidence, prevalence and mortality of CRC still remain high even in high-income countries. In particular, patients with stage 4 colorectal cancer at diagnosis have less than a 10% survival rate at 5 years owing to the ineffectiveness of current treatment regimens, which involve chemotherapy (for example, with 5-fluorouracil, oxaliplatin or irinotecan) and/or targeted therapy (for example, with bevacizumab, which targets vascular endothelial growth factor, or cetuximab, which targets epidermal growth factor receptor), optionally combined with radiation therapy for late-stage and advanced metastatic disease[1].

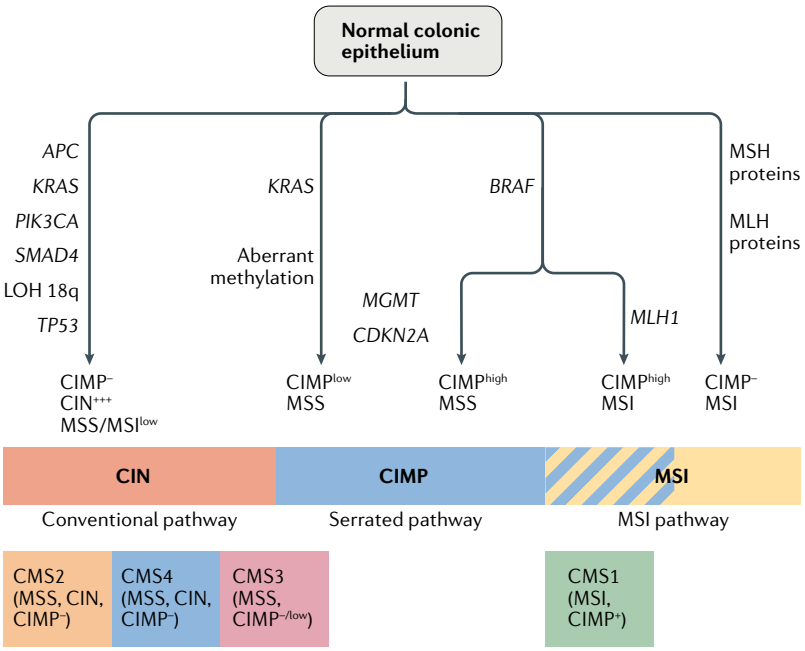
At least three major molecular pathways can lead to CRC (BOX 1). The most common, occurring in 70% of cases, is the chromosomal instability pathway, which is characterized by aneuploidy or structural chromosomal abnormalities, frequent loss of heterozygosity at tumour suppressor gene loci, and chromosomal rearrangements. Typically, tumours with chromosomal instability have mutations in specific oncogenes and/or tumour suppressor genes such as *APC*, *KRAS*, *PIK3CA*, *BRAF*, *SMAD4* or *TP53* (REF.[2]). Microsatellite instability (MSI), which is caused by dysfunction of DNA mismatch repair (MMR) genes, comprises another important pathway to (often right-sided) CRC that is associated with genetic hypermutability. The CpG island methylation pathway (CIMP) is the third pathway to CRC. CIMP$^+$ tumours can be divided into two types: CIMP^{high} tumours are associated with *BRAF* mutations and *MLH1* methylation, whereas CIMP^{low} tumours are associated with *KRAS* mutations[3]. Because the definition of the three pathways is not mutually exclusive, tumours can potentially be characterized by features of multiple pathways. Approximately 30% of CRC cases develop via a serrated pathway that is associated with the activation of the MAPK pathway (the mutually exclusive presence of either *KRAS* or *BRAF* mutations) as well as the presence of CIMP mutations (CIMP^{low} or CIMP^{high}).

However, although the underlying genetic alterations of CRC have been well established (BOX 1), the mutational make-up of tumour cells alone does not enable us to subclassify tumour types or to accurately predict patient survival. Instead, colon carcinogenesis provides an excellent example of the fact that tumour evolution, in addition to the presence of several essential mutations, depends on a close interaction of mutagenized cells with their tumour microenvironment (TME) (FIG. 1). The TME is composed of three main cell types: cancer-associated fibroblasts, vascular cells and infiltrating immune cells. Increasing evidence highlights also the importance of nerves for carcinogenesis[4]. All of these cell types closely interact in a reciprocal manner and can, in a cell non-autonomous manner, positively or negatively control the proliferation, cell death, growth suppressor evasion, energy metabolism and immune evasion of tumour cells as well as having effects on angiogenesis and tumour cell invasion.

Thus, non-malignant cells of the tumour stroma substantially contribute to carcinogenesis and the clinical relevance of these stromal cells in CRC is nicely illustrated by two examples. First, the presence of tumour-infiltrating lymphocytes is associated with a better prognosis in advanced CRC[5]. It has been shown that T helper 1 (T_H1) cell-mediated immune responses and interferon-γ (IFNγ) levels in CRC tumours are associated with a favourable prognosis, whereas T_H17 cell-mediated immune responses correlate with a less favourable prognosis, thus highlighting the key role of the T cell response in restricting or driving tumour cell growth[6]. This has led to the development of an

Box 1 | The three major molecular pathways of colorectal cancer

The majority (70–80%) of cases of colorectal cancer (CRC) follow the conventional chromosomal instability (CIN) pathway, which is initiated by *APC* mutation, followed by mutations in *KRAS*, *PIK3CA* and *SMAD4*, loss of heterozygosity of chromosome 18 (LOH 18q) and *TP53* mutation (see the figure). CRC development via this route is associated with high levels of CIN (CIN^{+++}), microsatellite stability (MSS) and no or low levels of the CpG island methylation pathway ($CIMP^{-}$). Approximately 20–30% of cases of CRC develop via the serrated pathway, which can be subdivided into $CIMP^{low}$ MSS tumours (which are often associated with *KRAS* mutations), *BRAF*-mutant $CIMP^{high}$ MSS tumours or *BRAF*-mutant $CIMP^{high}$ microsatellite instability (MSI) tumours. Serrated tumours are frequently associated with silencing of *MGMT*, *CDKN2A* or *MLH1*. In addition to the CIN pathway and CIMP, the MSI pathway is a third pathway of CRC development caused by dysfunction of DNA mismatch repair genes such as those encoding MLH proteins or MSH proteins. Based on these molecular pathways, CRC can be subclassified into four consensus molecular subtypes: CMS1 (MSI, $CIMP^{+}$), CMS2 (MSS, CIN, $CIMP^{-}$; which has the best overall prognosis), CMS3 (MSS, $CIMP^{-/low}$) and CMS4 (MSS, CIN, $CIMP^{-}$; which has the worst prognosis). It should be noted that this scheme is simplified and summarizes only the major correlations; the definition of the three pathways is not mutually exclusive and tumours can be characterized by features of multiple pathways.



Mismatch repair
A cellular system that recognizes and corrects DNA errors resulting from wrongly paired DNA bases that occur, for example, during DNA replication, DNA recombination or DNA damage.

Serrated pathway
An alternative pathway of genetic alterations occurring independently of *APC* mutations that is often initiated by the activation of the RAS–RAF–MEK–ERK–MAPK axis and is frequently characterized by a CpG island methylation pathway phenotype and subsequently by high-level DNA microsatellite instability, especially when located proximally and associated with *BRAF* mutations.

'immunoscore' for CRC tumours based on the quantification of cytotoxic and memory lymphocyte populations that has a prognostic value superior to that of the commonly used AJCC/UICC TNM classification[7]. The immunoscore provides a value based not only on the density but also on the position of $CD3^{+}$ and $CD8^{+}$ lymphocytes in the core tumour and invasive margin[8]. Whereas the infiltration of cytotoxic T cells, T_H1 cells and memory T cells strongly correlates with a better prognosis in all cancer types, the prognostic value of other immune cell infiltrates (for example, regulatory T (T_{reg}) cells, T_H2 cells, T_H17 cells, macrophages, natural killer (NK) cells, myeloid-derived suppressor cells (MDSCs) and B cells) shows more variability between the stage and type of cancer and requires the assessment not only of cell types but also of their functionality[9,10]. Moreover, the formation of tertiary lymphoid structures, which are more frequently found in the invasive margin or the stroma rather than in the tumour core, is associated with a favourable outcome in CRC[11]. However, despite the undisputed importance of T cell infiltration for the prognosis of patients with both microsatellite stability (MSS) and MSI types of CRC, it is not sufficiently understood why mainly MSI tumours with MMR deficiency are susceptible to immune-checkpoint blockade[12].

A second example of the relevance of the TME is provided by recent efforts to establish a consensus molecular subtype (CMS) classification of CRC by analysing gene expression profiles from more than 4,000 CRC samples, which has led to the identification of four subtypes (CMS1–CMS4)[13] (BOX 1). CMS1 represents hypermutated MSI tumours that are highly immunogenic, have immune-cell infiltration and respond to checkpoint blockade therapies[12]. By contrast, CMS4 tumours have the worst prognosis of all the subtypes and are characterized by abundant mesenchymal stroma and strong transforming growth factor-β (TGFβ) signalling profiles[14,15]. Dysregulated TGFβ signalling induces cancer-associated fibroblasts that help mediate the local invasion, metastasis and immune evasion of tumour cells[16]. Importantly, the inhibition of TGFβ signalling in fibroblasts enables T cell infiltration and responsiveness to checkpoint blockade in MSS tumours (tumours with chromosomal instability)[17].

The reciprocal interaction of cells within the TME markedly influences all stages of tumorigenesis and the plasticity of both tumour cells and surrounding cells within the TME is driven to a large extent by inflammation[18]. So far, all CRC tumours that have been studied are associated with an inflammatory environment (either preceding tumorigenesis, tumour elicited or therapy induced) and, over the past 5 years, our knowledge of how tumour cells communicate with the TME to modulate inflammatory immune responses has significantly improved. Thus, a detailed understanding of the molecular and cellular basis of the immune pathogenesis of CRC will undoubtedly lead to the development of novel and more efficient multi-modal therapeutic strategies that go beyond conventional therapies targeting tumour cells alone.

Here, we first review the three forms of inflammation in CRC and describe the mechanisms through which chronic inflammation can initiate tumorigenesis and through which tumour-elicited and therapy-induced inflammation can promote CRC. Some of these mechanisms are also relevant to the formation of distant metastases[19,20] but we do not cover this aspect here. We further specify how tumour cells engage and drive the plasticity of stromal cells in the TME and how extrinsic factors, such as diet, the microbiota and the mycobiota, contribute to inflammation and tumorigenesis in CRC. We conclude by providing an outlook of how our understanding of the interplay between inflammatory immune responses and tumorigenesis can be used to develop new prevention and treatment strategies for CRC.

Three forms of inflammation in CRC

According to the time point at which inflammation affects CRC pathogenesis, three types can be distinguished: chronic inflammation that precedes tumorigenesis, tumour-elicited inflammation and therapy-induced inflammation (FIG. 2). They all have in common the

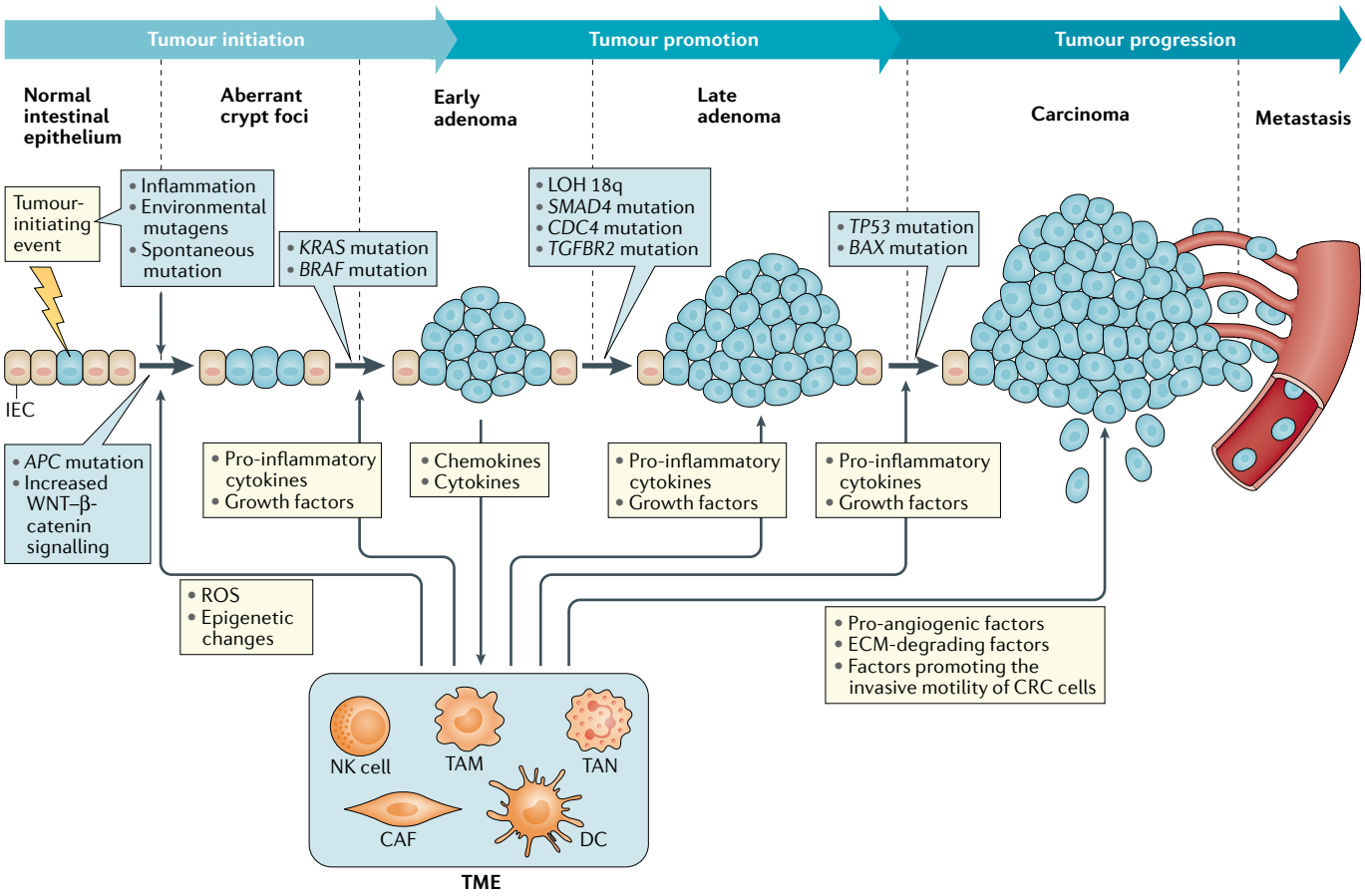


Fig. 1 | **Mechanisms of tumour initiation and promotion in colorectal cancer.** Colorectal tumorigenesis requires a tumour-initiating event that transforms normal intestinal epithelial cells (IECs) by spontaneous mutation, environmental mutagens or inflammation-induced (epi-)genetic changes. The clonal expansion of these 'initiated' cells — driven by mutations that cause hyperproliferation, such as of *APC* or other genes encoding signalling components of the WNT pathway, additional mutations such as of *KRAS*, *TP53* or *TGFBR2*, and growth stimulatory factors from the tumour microenvironment (TME) — leads to the outgrowth of these clones into malignant tumours, which is known as tumour promotion. Further mutations and changes to the TME enable these tumours to later metastasize to distant organs. The epithelial tumour tissue is in constant interaction with cells in the TME through the effects of cytokines, chemokines and growth factors. An inflammatory environment not only contributes to tumour initiation, for example, through the production of reactive oxygen species (ROS) or epigenetic changes, but also promotes tumorigenesis by providing growth factors and pro-inflammatory cytokines. Furthermore, growing tumours can stimulate an inflammatory TME through the production of cytokines and chemokines and thereby create a positive-feedback loop that drives tumour progression. CAF, cancer-associated fibroblast; CRC, colorectal cancer; DC, dendritic cell; ECM, extracellular matrix; LOH, loss of heterozygosity; NK, natural killer; TAM, tumour-associated macrophage; TAN, tumour-associated neutrophil.

AJCC/UICC TNM classification
The AJCC/UICC staging system is a classification system developed by the American Joint Committee on Cancer and the Union Internationale Contre le Cancer for describing the extent of disease progression in patients with cancer using a system that scores for tumour size, lymph nodes affected and the presence of metastases.

Myeloid-derived suppressor cells
(MDSCs). A heterogeneous group of cells from the myeloid lineage that have immunosuppressive properties.

tumour-promoting activation of innate immune cells and the establishment of a immunosuppressive TME.

Inflammation-associated tumorigenesis. Chronic inflammation triggered by infections, aberrant immune reactions or environmental factors, such as tobacco smoking, inhaled pollutants or dietary factors, markedly increases the risk of tumour development[21]. In the context of CRC in particular, long-standing and poorly controlled inflammatory bowel disease (IBD) as well as chronic inflammation of the gastrointestinal tract induced by poor dietary habits, such as a western-style diet, are the main risk factors[22,23]. Although only up to 5% of all CRC tumours develop in the context of overt chronic inflammation, mouse models of inflammation-associated tumorigenesis, particularly the azoxymethane/dextran sulfate sodium model (AOM/DSS model) (TABLE 1), have been very informative for the identification of various mechanisms of tumorigenesis, many of which have also been shown to be of relevance to sporadic tumorigenesis.

Two subsequent events are required to induce tumour formation from normal cells. First is a tumour-initiating event that requires an accumulation of mutations or epigenetic alterations, leading to either the inactivation of tumour suppressor genes or the hyperactivation of oncogenes that provide cells with growth and survival advantages. Second is tumour promotion, which involves the clonal expansion of cells harbouring those mutations and outgrowth of those clones into a frank tumour. Inflammation strongly contributes to both of these key events.

Inflammation can initiate tumorigenesis via DNA damage in the absence of any exogenous carcinogens[24]. This might in part be attributed to increased oxidative stress, caused by tissue-resident or recruited cells of the innate immune system (such as macrophages and neutrophils) that release increased levels of reactive oxygen

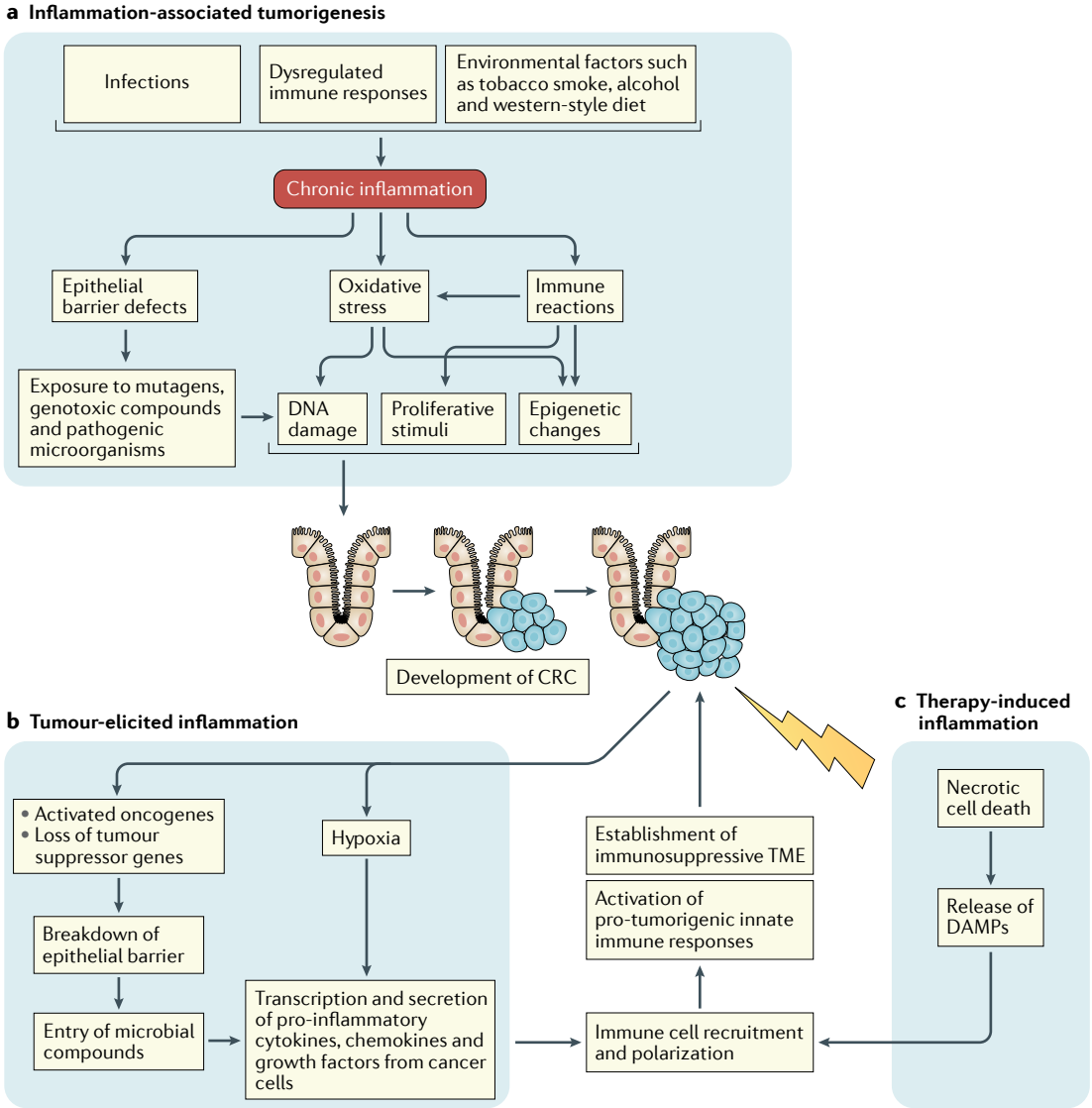


Fig. 2 | **The inflammatory environment of colorectal cancer.** There are three main ways in which inflammation can be associated with colorectal cancer (CRC). **a** | In inflammation-associated tumorigenesis, chronic inflammation resulting from infections, dysregulated immune responses or environmental factors can initiate and promote tumorigenesis through the induction of DNA damage or epigenetic changes, for example, caused by oxidative stress or exposure of the stem cell compartment to mutagenic compounds owing to epithelial barrier defects, or through constant exposure to inflammatory proliferative stimuli. **b** | In tumour-elicited inflammation, tumour progression initiates an inflammatory response that is often pro-tumorigenic owing to hypoxia-induced cell death or to breakdown of the epithelial barrier and the subsequent influx of microbial products. **c** | Similarly, therapy-induced inflammation can trigger tumour-promoting inflammation owing to the release of damage-associated molecular patterns (DAMPs) from necrotic cells. TME, tumour microenvironment.

Immune-checkpoint blockade
A therapeutic concept that aims to block negative feedback signalling to immune cells to enhance an immune response against tumours.

AOM/DSS model
A mouse model of inflammation-associated colorectal cancer, in which intestinal tumorigenesis is triggered by injection of the mutagen azoxymethane (AOM) and subsequent cycles of inflammation induced by dextran sulfate sodium (DSS).

and nitrogen species into the tissue microenvironment during inflammation. Reactive oxygen and nitrogen species can cause various kinds of DNA damage in intestinal epithelial cells (IECs), including single-strand and double-strand breaks, nucleotide modifications and abasic sites. Indeed, the increased production of reactive oxygen species by myeloid cells can induce genome-wide DNA mutations and directly transform IECs to initiate tumorigenesis during chronic intestinal inflammation without any carcinogenic treatment[25]. Furthermore, intestinal inflammation affects epithelial barrier function and can expose the intestinal stem cell compartment to environmental mutagens or bring stem cells in close vicinity to active inflammatory cells that release genotoxic compounds[26].

In addition, the breakdown of the intestinal barrier triggers the invasion of commensal and pathogenic microorganisms and leads to contact between IECs and components of the microbiota that might have pro-tumorigenic characteristics. Chronic intestinal inflammation leads to excessive tissue regeneration, triggering the proliferation and clonal expansion of initiated tumour cells (tumour promotion) as well as the dedifferentiation of non-stem cells into stem-like

Table 1 | **Selected current mouse models of colorectal cancer**

Mouse model	Purpose of study	Primary tumour location	Invasion	Metastasis	Effect of intestinal microbiome on phenotype	Advantages	Disadvantages	Example references
AOM-based models								
AOM	Sporadic tumorigenesis	Distal colon	No	No	Yes	Reliable and easy to perform	Relatively long term (4–5 months); no genetically defined mutations	162
AOM in $p53^{\Delta IEC}$ or $p53^{\Delta IEC/AktE17K}$	Sporadic tumorigenesis	Distal colon	Yes	Lymph node and liver	Yes	Reliable and easy to perform	Relatively long term (4–5 months)	48,65
AOM/DSS	Inflammation-associated tumorigenesis	Distal colon	No	No	Yes	Cheap and efficient model to study CRC driven by chronic inflammation	Relatively long term (3–4 months); no genetically defined mutations	163
Genetically engineered models								
$Apc^{Min/+}$	APC-driven tumorigenesis, model for FAP	Small intestine	No	No	Yes	Fast development of multiple adenomas; no additional treatment necessary	No colonic tumours; no possibility to study tumour progression as animals die at the adenoma stage	164
$Apc^{Min/+}$; $Trp53^{-/-}$	APC/p53-driven tumorigenesis	Small intestine	Yes	No	Yes	Fast development of colonic and small intestinal tumours; adenocarcinoma development	Requires breeding of genetically engineered mice	165
CDX2-Cre; APC^{fl}	APC-driven colon-specific tumorigenesis	Colon	No	No	Yes	Fast model to study tumour onset from colonic cells	Requires breeding of genetically engineered mice	166
$Apc^{lox/lox}$; $Trp53^{lox/lox}$; Tet-O-LSL-$Kras^{G12D}$; $VillinCre^{ERT2}$	APC/p53/KRAS-driven tumorigenesis and metastasis	Colon	Yes	Lymph node, liver, lungs	Yes	Good model to study aggressive CRC and metastasis; mutations are IEC specific	Requires breeding of genetically engineered mice	167
Xenotransplant models								
Subcutaneous injection of tumour cells or tumour organoid material	Primary tumour growth	Injection site	Depends on aggressiveness of starting material	No (mice are sacrificed before metastasis)	No	Fast and easily achievable; easy to measure tumour growth	Neglects complexity of tumour development and metastasis; no involvement of TME	168
Transplantation of organoids								
Mucosal injection of $Apc^{flox/flox}$; $Kras^{LSL-G12D}$; $Trp53^{flox/flox}$ mouse organoids or PDX organoids	Tumour progression, invasion and metastasis on site	Colon	Yes	Depends on mutational profile of organoids	Yes	Single tumours in distal colon in immunocompetent mice; tumour progression in the correct microenvironment; only tumour tissue harbours mutations; relatively quick model (4–6 weeks)	Submucosal injection might bypass first metastatic steps; requires training and special equipment	169
Organoid transplantation in DSS-injured colonic tissue	Tumour progression, invasion and metastasis on site	Colon	Yes	Depends on mutational profile of organoids	Yes	Single tumours in distal colon in immunocompetent mice; tumour progression in the correct microenvironment; only tumour tissue harbours mutations; relatively quick model (4–6 weeks)	Difficult to titrate correct amount of DSS to induce sufficient extent of colitis	170

AOM, azoxymethane; CRC, colorectal cancer; DSS, dextran sulfate sodium; FAP, familial adenomatous polyposis; IEC, intestinal epithelial cell; PDX, patient-derived xenograft; TME, tumour microenvironment.

cells to cope with the regeneration of the damaged tissue[27–29] (FIG. 2a). Intestinal stem cells are much better equipped than non-stem cells to resist high levels of replication stress and of DNA-damaging agents, and de-differentiating post-mitotic cells have indeed been shown to acquire a tumour-initiating capacity. Thus, inflammation can increase the mutational burden as well as the number of potentially tumour-initiating cells[30]. Furthermore, inflammation affects key cytokine receptor-mediated signalling pathways that control central tumour-initiating and tumour-promoting processes in CRC such as the activation of nuclear factor-κB (NF-κB) downstream of tumour necrosis factor (TNF) receptor and IL-1 receptor signalling[31–34] as well as the gp130-dependent activation of the transcription factor STAT3 downstream of IL-6-induced and/or IL-11-induced signalling[35–37]. By contrast, IL-22, another STAT3-activating cytokine, triggers the transcription of DNA damage response genes to counteract inflammation-induced genotoxic effects[38]. Notably and different to sporadic CRC, in inflammation-driven CRC, gain-of-function mutations in *TP53* are detected very early[39] and are known to enhance TNF, NF-κB and STAT3 signalling[40,41]. Other somatic gene mutations that have recently been identified in inflammatory conditions such as human ulcerative colitis, including *NFKBIZ*, *ZC3H12A*, *TRAF3IP2* and *HNRNPF* mutations, are only infrequently found in CRC[42,43].

Besides causing mutations through DNA damage, inflammation can also affect cancer-related genes through epigenetic mechanisms that result in silencing of important tumour suppressor genes[44]. For example, IL-1β, IL-6 and TNF control the expression of the DNA methyltransferases DNMT1 and DNMT3, thereby leading to changes in the methylation and expression pattern of genes involved in CRC pathways such as NOTCH or p53 signalling[45,46]. Of note, NOTCH signalling has recently been shown to lead to highly penetrant metastasis in *KRAS*-driven CRC through TGFβ-dependent neutrophil recruitment[47] and to drive invasion and metastasis in a CMS4 tumour model[48].

Prostanoids are lipid mediators that are produced during chronic inflammation in a cyclooxygenase 1 (COX1)-dependent and COX2-dependent manner and that have a major impact on all stages of colorectal tumorigenesis. For example, prostaglandin E_2 (PGE_2), the most abundant prostanoid in CRC, promotes tumour initiation and growth by inducing the expression of DNMT1 and DNMT3B and the clonal expansion of cancer stem cells by activating NF-κB signalling[49]. Furthermore, PGE_2 induces the differentiation of MDSCs and T_{reg} cells[50], affects macrophage polarization[51], and inhibits the NK cell-dependent remodelling of the TME to enable immune evasion[52].

Additional mechanisms to explain how inflammation can exert the epigenetic silencing of cancer-related pathways involve the actions of microRNAs and long non-coding RNAs (lncRNAs) targeting the WNT pathway and the Hippo pathway — two important drivers of CRC[53] — or regulating various key signalling pathways involved in CRC such as p53, NF-κB and STAT3 signalling[54–58]. For example, levels of the lncRNA H19 are increased in patients with ulcerative colitis as well as in mouse models of lipopolysaccharide-induced sepsis and DSS-induced colitis[59]. During inflammation, IL-22 produced by innate lymphoid cells, neutrophils, T_H17 cells and T_H22 cells[60–62] can induce the expression of H19 lncRNA in IECs, which enhances their proliferation by functioning as a competitive inhibitor of growth inhibitory microRNAs and thus increases the speed of regeneration of the colonic epithelium during DSS-induced colitis[59]. Similarly, the IL-22-mediated increase of H19 lncRNA might enhance the proliferative capacity of malignant cells during the course of colitis-driven CRC.

Tumour-elicited inflammation. Although inflammation can be a potent driver of tumour initiation, the vast majority of cancers are not preceded by overt inflammation. Nevertheless, sporadic tumours can elicit inflammation and they depend strongly on the cell–cell interactions within the TME to promote local tumour growth as well as the formation of distant metastases[18] (FIG. 2b). Several mechanisms that modulate innate and adaptive immune responses and stromal cell activation during CRC pathogenesis have been discovered. One of the first events comprises a loss of intestinal barrier function upon WNT activation in IECs, for example as a result of *APC* mutation, which enables microbial products from the intestinal lumen to access and activate tissue-resident IL-23-expressing myeloid cells, leading to the release of IL-17A, which is a promoter of early CRC[63]. IL-1, expressed by monocytes, stromal cells and tumour epithelial cells, is another crucial mediator of tumour-elicited inflammation and acts on diverse cell types simultaneously[64]. Whereas IL-1 signalling in IECs promotes tumorigenesis, NF-κB activation and cell proliferation independently of tumour-elicited inflammation, IL-1 signalling in T cells activates IL-23 expression and drives inflammation to promote tumorigenesis. Notably, myeloid cell-specific IL-1 signalling controls tumour infiltration by microorganisms and is crucial for preventing tumour-associated dysbiosis and tumour-elicited inflammation after breakdown of the epithelial barrier[64]. Moreover, the loss of p53 function at later stages of tumorigenesis impairs epithelial integrity to fuel microbial activation of NF-κB and STAT3 pathways and hence inflammation[65]. Thus, oncogene activation and tumour suppressor loss not only promote cancer cell survival and proliferation in a cell-autonomous manner but also sustain tumour-elicited inflammation by indirectly inducing the production of pro-inflammatory cytokines, growth factors and chemokines that recruit inflammatory immune cells to the tumour site[21].

In addition, hypoxia and a lack of sufficient nutrients in growing tumours whose size exceeds their blood supply lead to necrotic cell death and to the secretion of pro-inflammatory damage-associated molecular patterns such as HMGB1, IL-1, uric acid or ATP[66,67]. Tumour hypoxia can induce the expression of hypoxia-inducible factor 1α (HIF1α) in tumour cells as well as in cells within the TME and can activate cancer-associated fibroblasts to release TGFβ, CXCL13 and other chemokines that recruit diverse myeloid and

WNT pathway
An evolutionarily conserved pathway that is important for embryonic development and carcinogenesis, which is activated in more than 90% of colorectal cancers owing to mutations in its signalling components.

lymphoid cells to the TME, including monocytes, macrophages and B cells in prostate cancer[68]. Furthermore, in the presence of increased TGFβ levels, hypoxic stress increases T_{reg} cell formation and suppresses the differentiation of effector T cells, thus impairing anti-tumour immune responses in CRC[69].

Importantly, the cytokine milieu in the colorectal TME triggers the activation of a tumour-promoting inflammatory response but also shapes an immunosuppressive phenotype. For example, tumour-derived cytokines such as TGFβ attenuate the antigen presentation function of dendritic cells (DCs), inhibit T cell proliferation and effector function, induce the formation of T_{reg} cells, and inhibit the function of cytotoxic NK cells[70]. Increased levels of TGFβ in the TME also drive immune evasion via T cell exclusion; the inhibition of TGFβ signalling enabled immune cell infiltration into MSS-like CRC tumours, which resulted in a strongly increased response to immune-checkpoint blockade and to the eradication of established metastases[17]. Human serrated CRC tumours have reduced expression of atypical protein kinase Cs (PKCς and PKCλ/ι), which is associated with impaired immune surveillance[71]. Interestingly, in these tumours, inhibiting TGFβ in combination with checkpoint blockade is very effective in reducing the load and aggressiveness of tumours. Another mechanism by which tumour cells can directly evade T cell recognition is through the suppression of STAT3-dependent mitophagy in IECs, which is involved in antigen processing[72]. Moreover, the recruitment of B cells into the TME can have immunosuppressive effects. The exposure of B cells to TGFβ and other cytokines (IL-21, IL-33 and IL-10) in the TME triggers class switching from IgM to IgA[73–75]. The primary role of IgA is to maintain homeostasis with the microbiota, which is in general anti-tumorigenic; in addition, IgA^+ plasma cells have anti-inflammatory and immunosuppressive properties[76]. For example, in the liver, chronic inflammation leads to an accumulation of IgA^+ plasma cells that express PDL1 and IL-10 and directly suppress cytotoxic $CD8^+$ T cells and, thereby, anti-tumour immunity[75]. An increased number of IgA-secreting B cells can also be found in patients with CRC[77], although direct mechanistic studies analysing the function of B cells and/or IgA in CRC are still lacking.

Therapy-induced inflammation. Another type of inflammation with a marked impact on the course of CRC is therapy-induced inflammation (FIG. 2c). Radiotherapy and chemotherapy induce tumour cell death as well as alterations within the TME, thereby inducing cellular 'wound-healing' responses[21]. Therapy-induced inflammation is an often-unwanted consequence of therapy rather than the aim but it is nevertheless an important determinant of therapeutic response and relapse that can have both anti-tumorigenic and pro-tumorigenic effects depending on the context. Dying tumour cells can release damage-associated molecular patterns (including ATP, double-stranded DNA, calreticulin and HMGB1) that recruit and activate antigen-presenting cells. Combined with the enhanced release of tumour neoantigens from dying cells, this might activate de novo T cell responses and improve immunosurveillance[78].

However, accumulating experimental and clinical data show that dying tumour cells can also promote tumorigenesis and suppress anti-tumour immunity. For example, IL-1α released by necrotic cells can promote malignant transformation as well as angiogenesis and metastasis. In addition, IL-1α activates and polarizes fibroblasts towards an inflammatory phenotype, which may ultimately promote tumorigenesis[79]. Furthermore, radiotherapy and chemotherapy have been shown to have immunosuppressive effects. Ionizing radiation can increase the number of T_{reg} cells in skin tumours[80] and the chemotherapy drug oxaliplatin can recruit immunosuppressive plasma cells to malignant tissues in prostate cancer[74]. Moreover, the therapy-induced death of tumour cells can induce the production of growth factors and cytokines, such as WNT, epidermal growth factor, TNF, IL-17 and IL-6, by cells of the TME, which promote the survival of the remaining tumour cells and contribute to therapy resistance[18].

One distinct form of therapy-induced inflammation is the targeted activation of the immune system by checkpoint blockade, which is the main pillar of current immunotherapies[81]. PDL1 and CTLA4 immune-checkpoint inhibitors are partially effective in patients with metastatic MMR-deficient/MSI^{high} CRC[82,83]. However, not all MSI tumours respond well to immunotherapy and they can develop immune evasion mechanisms that are frequently associated with loss-of-function mutations in genes involved in HLA class I-restricted antigen presentation[84,85]. Surprisingly, MMR-proficient/MSS CRC, which accounts for most metastatic tumours, is less responsive to immune-checkpoint blockade, which is in contrast to the predictive value of the immunoscore for the overall prognosis of patients with CRC irrespective of the microsatellite status. This might be explained by the downregulation of HLA class I and class II molecules, increased infiltration by MDSCs, low levels of inhibitory receptor expression (such as PD1)[86], and the polarization of macrophages and cancer-associated fibroblasts towards an immunosuppressive state in MMR-proficient/MSS CRC. However, the elucidation of the exact underlying mechanisms remains a key task in the field.

Inflammation-driven cellular plasticity

In general, it is now well accepted that tumours are a composite of genetically and epigenetically heterogeneous cancer cells and that they differ markedly in terms of the quantity and phenotypes of immune and stromal cells in the TME owing to a high degree of phenotypic plasticity within the TME. Colorectal tumorigenesis relies greatly on the plasticity of both tumour and surrounding cells, which is driven to a large extent by inflammation[18].

One of the most plastic cell types in the TME are cancer-associated fibroblasts[87]. Fibroblasts are responsible for most of the extracellular matrix protein production and fibrillogenesis within the TME and they further contribute, together with tumour cells, to extracellular matrix stiffness, to stimulating stemness, cancer cell survival, growth and invasion, and to angiogenesis by releasing pro-angiogenic factors. Importantly, they have

Mitophagy
Selective degradation of mitochondria by autophagy.

a major impact on the generation of a pro-inflammatory milieu and the polarization of immune cells and, by creating a physical barrier, they significantly contribute to T cell exclusion from the tumour, thereby affecting every aspect of multi-step tumorigenesis[79]. Recently, single-cell analyses of CRC have revealed fibroblast subsets that are characterized by distinct transcriptomes and secretory profiles. Fibroblasts with inflammatory or myofibroblastic characteristics could be identified in pancreatic, breast and colorectal cancers that are polarized, for example, by inflammatory signals such as IL-1 and that typically secrete high levels of CXCL1 and IL-6 or TGFβ, respectively[88–90]. Although these results highlight the enormous heterogeneity of cancer-associated fibroblasts, their functional contribution to CRC pathogenesis and therapeutic response remains unclear thus far.

Furthermore, myeloid cells show a high degree of plasticity driven by signals in the TME[91]. For example, tumour-associated macrophages (TAMs), which are the most common myeloid cell type in the TME, derive from monocytic precursors that are recruited to the tumour tissue by chemokines released from cancer cells. In the past, macrophages have been classified based on their in vitro properties into two distinct subtypes: inflammatory M1-type macrophages, which have pronounced anti-tumour activity, and M2-type macrophages, which are pro-tumorigenic and immune suppressive. However, it is now recognized that macrophages can move through a spectrum of functional phenotypes in response to environmental cues in vivo[92]. Although, the pro-tumorigenic activity of TAMs during CRC development has been shown in several studies, their contribution to CRC progression is controversial. In contrast to many other solid cancers, in which TAM infiltration is associated with worse prognosis, TAM infiltration in CRC has been unable to predict outcome[93] or has been correlated with a better prognosis[94]. This might be due to the simultaneous accumulation of M1-like and M2-like macrophages or to the spatial distribution of TAMs within the tumour (with evidence that TAMs at the invasive front are anti-tumorigenic, whereas TAMs in the central tumour are pro-tumorigenic) or it might depend on the MSI subtype or on type of treatment (for example, TAM infiltration usually correlates with a poor outcome in stage 3 CRC but correlates with overall survival in patients who received 5-fluorouracil adjuvant therapy)[95,96]. Interestingly, even simple differences in TAM morphology correlate with functional diversity and prognostic significance in patients with CRC liver metastasis, as small TAMs are associated with better overall survival than large TAMs[97].

Neutrophils are also recruited to the inflamed TME and, by analogy to M1-like and M2-like macrophages, tumour-associated neutrophils (TANs) can have tumour-suppressive (N1) or tumour-promoting (N2) subtypes[98–100]. N1 TANs express pro-inflammatory factors and have a greater ability to kill tumour cells in vitro, whereas N2 TANs have a reduced expression of immunoactive factors and produce large amounts of arginase, which suppresses T cell function. Usually, tumour-suppressive N1-like subtypes are found in the early stages of cancer but transform into a tumour-promoting N2-like subtype as tumours progress[101]. TGFβ has been shown to be a major player in reprogramming N1-like TANs towards N2-like TANs, whereas a combination of IFNγ and GM-CSF can polarize neutrophils towards an N1-like subtype[102,103]. Moreover, the formation of neutrophil extracellular traps (NETosis), which is presumably associated with N2-like TANs, correlates with worse prognosis in patients with CRC[104] likely by affecting immunoediting (for example, by forming barriers between tumour cells and cells of the immune system) and metastatic spread by the proteolytic degradation of the extracellular matrix[105]. Importantly, an increased ratio of neutrophils to lymphocytes has been correlated with a worse prognosis in patients with advanced CRC[106]. However, the precise role of TANs in CRC is not yet fully elucidated and there is as yet no strategy available to specifically divide immunosuppressive TANs from other TAN subtypes[99]. Furthermore, both the M1/M2 and N1/N2 classifications should only be used for in vitro-differentiated cells as, in vivo, both macrophages and neutrophils are highly specialized, transcriptomically dynamic and extremely heterogeneous with regards to their phenotypes and functions, which are continuously shaped by their tissue microenvironment[107].

The potent effects of the TME on the cellular plasticity of tumour and stromal cells as well as on the exclusion or recruitment of certain immune cells have a marked impact on tumour progression and response to therapy. The polarization profiles of cancer-associated fibroblasts and myeloid cells are not determined by distinct transcription factors as is the case for T cells. Consequently, they can easily respond and adapt to changes in the cytokine milieu, thereby rendering these cells very responsive to therapeutic interference and re-polarization. Therefore, cancer-associated fibroblasts and myeloid cells are extremely attractive target cell populations for therapies that aim to enable or improve standard cytotoxic therapies or immunotherapies.

Extrinsic factors and inflammation

In the past few decades, several extrinsic risk factors that increase or decrease the risk of developing CRC have been described.

Diet. The incidence of CRC is highest in 'westernized' countries, such as the United States, Australia and in Europe, but incidence is now also rapidly increasing in line with economic development in other parts of the world. Besides the increased life expectancy and demographic ageing in higher-income countries, environmental factors and lifestyle are the main factors thought to be responsible for the increased risk of developing CRC. There are numerous environmental factors that predispose to CRC, either directly through exposure to mutagens (for example, *N*-nitroso compounds, polycyclic aromatic hydrocarbons and heterocyclic amines from processed meat, tobacco smoke or air pollutants) or indirectly through repeated or chronic exposure to inflammatory stimuli, mostly of low grade and intensity. Diet is recognized as being one of the most important extrinsic risk factors for CRC through its effects

Immunoediting
Evolution of tumours with the result that tumour cells are no longer effectively recognized and suppressed by the immune system, resulting in the emergence of immune-resistant tumour cell variants.

on intestinal tissue homeostasis, intestinal immunity and the intestinal microbiome. Both malnutrition and obesity have marked effects on inflammatory and immune responses[108]. High-fat 'western style' diets have been shown not only to accelerate tumorigenesis in the AOM/DSS model and in *Apc*-mutant models of CRC but also to spontaneously induce CRC[27]. Importantly, the tumour-promoting effects of such diets are not necessarily linked to obesity or caloric intake[109]. Genetically induced obesity and diet-induced obesity have different effects not only on microbiome composition but also on cytokine production and can thus independently increase inflammation and CRC risk[110]. In general, obesity causes chronic subclinical inflammation through various mechanisms, resulting in skewed macrophage polarization[111] and immunosuppression. For example, obesity results in decreased DC function, T cell exhaustion and reduced numbers of NK cells[112]. Accordingly, in renal cell carcinoma, immunotherapy targeting DCs, which has beneficial effects in lean mice, has not shown efficacy in obese mice[113].

Paradoxically, obesity is associated with an increased efficacy of immune-checkpoint blockade through PD1–PDL1 in mouse models of melanoma and in patients with several other types of cancer[114]. There is as yet no clear explanation for this paradox; however, it is likely that the energy balance of tumour cells within the TME might underlie the observed interaction between body mass index and immune therapy. For example, in renal cell carcinoma, alterations in fatty acid metabolism were associated with both a high body mass index and improved treatment outcomes[115]. In line with this, patients with obesity who were treated with immunotherapy for metastatic melanoma had a significantly improved progression-free and overall survival[116]. Besides favouring obesity through overnutrition, a 'western-style' diet also modulates the gut microbiome, which can have marked effects on host metabolism, intestinal stem cell proliferation and function of the immune system. However, the gut microbiome is also influenced by many other and incompletely understood factors such as host genetics, age, gender or geography[117].

The microbiome. A healthy microbiome is essential for the development of the immune system and for coordinating immune responses, whereas dysbiosis is associated with various diseases, including IBD and CRC. Accordingly, the composition of mucosa-adhering bacteria from healthy control tissue differs markedly from the bacterial composition of tissues from patients with CRC[118]. The evaluation of stool samples from healthy individuals, patients with IBD and patients with CRC has revealed specific bacterial strains that correlate with the pathology of the disease[119–121].

Various mechanisms could explain how bacteria contribute to the development of CRC (FIG. 3a). First, bacteria and their metabolites can be genotoxic and transform IECs directly. The most prominent example of this is *Escherichia coli* expressing the genomic island polyketide synthase (pks$^+$ *E. coli*), which produce colibactin that generates potentially mutagenic DNA adducts[122]. Pks$^+$ *E. coli* are enriched in preclinical models of CRC and in human tumour tissues[123] and they induce a unique mutational signature in ex vivo-cultured, untransformed colon organoids that can also be detected in patients with CRC[124]. Moreover, the microbial production of gallic acid can locally influence the activity of mutant tumour suppressor proteins, such as p53, in intestinal epithelia, which indicates that the oncogenic capacity of a mutation can be controlled by the microbiome[125].

Second, the loss of surface barrier function in CRC tumours can trigger commensal bacteria-induced, tumour-promoting inflammation. For example, the disruption of tight junctions between colonic tumour cells allows for the degradation products of commensal bacteria, such as lipopolysaccharide, to enter the tumour stroma, which then leads to the recruitment of myeloid cells into the TME. In addition, commensal bacteria themselves can invade the tumour tissue and induce tumour-infiltrating myeloid cells to produce inflammatory cytokines and promote CRC tumorigenesis.

Third, pathogenic bacteria can induce colonic inflammation and hence tumorigenesis. For example, *Fusobacterium nucleatum* is an anaerobic Gram-positive oral commensal bacterium that is usually not found elsewhere in the body under normal conditions. However, human CRC tissues are enriched for *F. nucleatum*[126,127], which correlates with worse prognosis[128]. *F. nucleatum* promotes tumorigenesis by acting on both tumour cells and immune cells in the TME. For example, *F. nucleatum* activates β-catenin signalling in colonic epithelial cells, which in turn upregulates the expression of oncogenes, such as those encoding cyclin D1 and MYC, and pro-inflammatory signals such as TNF and IL-17 (REF.[129]). In the *Apc*$^{Min/+}$ mouse model, *F. nucleatum* colonization increases the number of CRC tumours and recruits MDSCs, TANs, TAMs and immature DCs with immunosuppressive functions, which collectively promote tumour progression[130]. Furthermore, *F. nucleatum* binds to the immune inhibitory receptor TIGIT to suppress T cell activation and NK cell-mediated killing of colonic tumour cells[131]. *F. nucleatum* also induces expression of the chemokines CXCL8 and CXCL1, thereby promoting invasive motility and increasing the metastatic potential of infected and non-infected tumour cells[132]. Unlike *F. nucleatum*, which can promote tumorigenesis regardless of colitis development, enterotoxigenic *Bacteroides fragilis* (ETBF) initially induces colitis and then subsequently tumorigenesis[133]. ETBF also recruits other bacteria to create biofilms that coat adenomas and CRC tumours[134], where they trigger a pro-inflammatory cascade in colonic epithelia to promote CRC[135]. Furthermore, ETBF can recruit MDSCs to the TME, further contributing to the suppression of anti-tumour immune responses in CRC[136].

Finally, bacteria, together with the specific dietary components, can regulate the immune system to favour or prevent intestinal inflammation and tumorigenesis through the production of metabolites or co-metabolites[137]. The gut microbiome produces a diverse metabolite repertoire from the fermentation of exogenous dietary compounds or endogenous microbial or host compounds present in the intestinal lumen.

Apc-mutant models
Mouse models of intestinal cancer in which tumorigenesis is driven by a loss of function of APC, resulting in the hyperactivation of the WNT pathway and the hyperproliferation of affected cells.

Apc$^{Min/+}$ mouse model
A mouse model of intestinal cancer in which the *Apc* gene has a truncation mutation at codon 850, resulting in multiple intestinal neoplasia (Min).

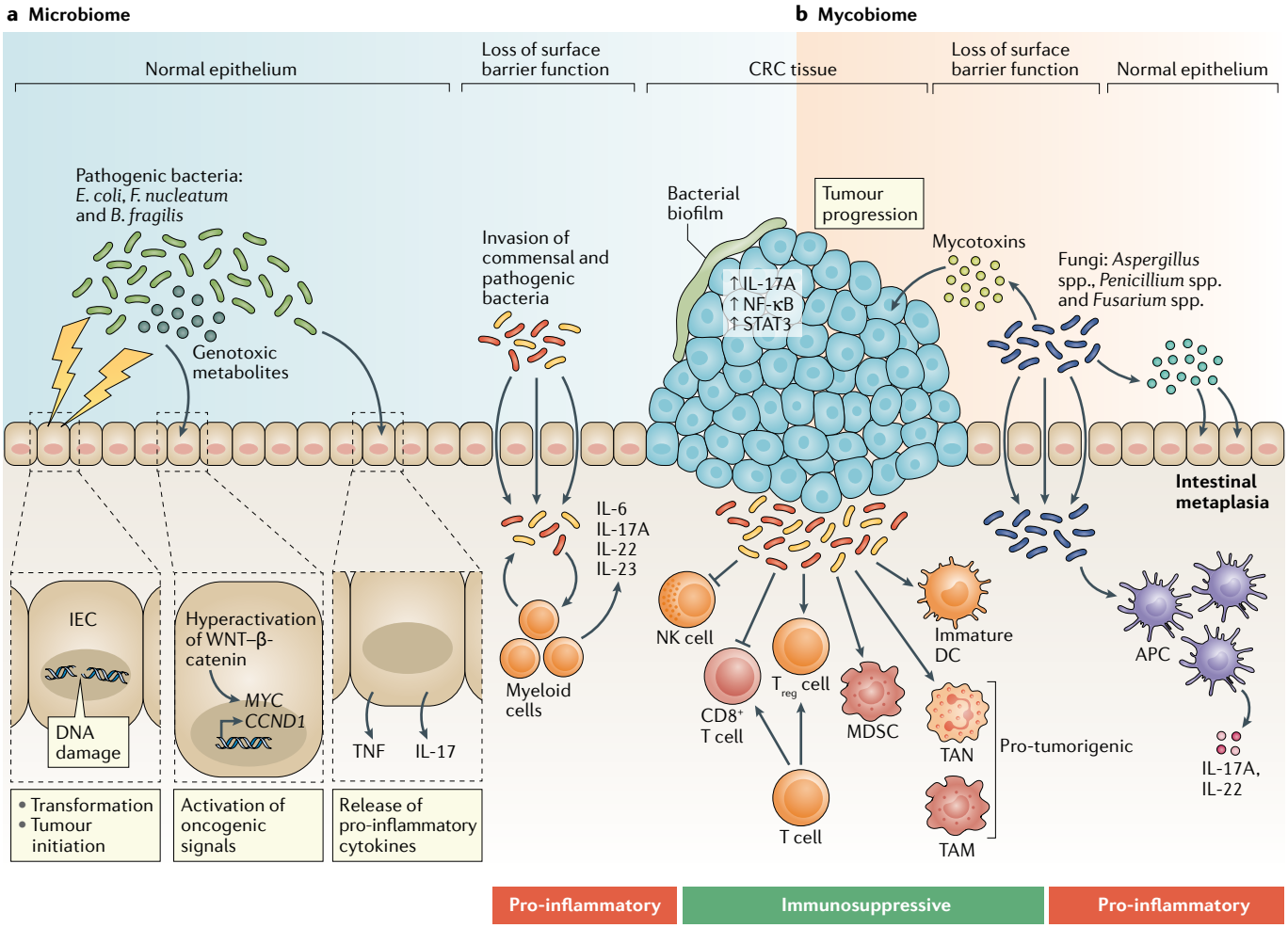


Fig. 3 | **The roles of the microbiome and mycobiome in colorectal cancer.** **a** | Pathogenic bacteria, such as *Escherichia coli*, *Fusobacterium nucleatum* and *Bacteroides fragilis*, and their metabolites can induce tumorigenesis through either direct mutagenic effects on intestinal epithelial cells (IECs) or the activation of intracellular oncogenic signals, for example hyperactivation of the WNT–β-catenin pathway. Furthermore, bacterial metabolites can trigger the release of pro-inflammatory signals, such as tumour necrosis factor (TNF) and IL-17, that can further promote tumorigenesis. In addition, loss of surface barrier function can lead to the invasion of commensal and pathogenic bacteria from the intestinal lumen, which can induce a tumour-promoting inflammatory response by myeloid cells. Bacteria can form tumour-coating biofilms that trigger pro-inflammatory signalling cascades (involving nuclear factor-κB (NF-κB), STAT3 and IL-17 receptor A (IL-17RA)) that promote colorectal cancer (CRC). In addition, bacteria can lead to immunosuppression in the tumour microenvironment by recruiting immunosuppressive cells (such as myeloid-derived suppressor cells (MDSCs) and regulatory T (T_{reg}) cells) or by polarizing immune cells towards pro-tumorigenic and/or immunosuppressive phenotypes (such as tumour-associated macrophages (TAMs) and tumour-associated neutrophils (TANs)). **b** | The mycobiota, including *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp., might also affect colorectal tumorigenesis. On the one hand, mycotoxins can be directly carcinogenic and induce intestinal metaplasia; on the other hand, fungi detected by the immune system can induce a pro-tumorigenic inflammatory environment, for example involving the secretion of IL-17A and IL-22. Both the microbiota and mycobiota can also suppress immunity by reducing the levels of pro-inflammatory cytokines such as TNF and IL-6 or by recruiting immunosuppressive cells. APC, antigen-presenting cell; DC, dendritic cell; NK, natural killer.

Once these compounds pass the mucosal barrier, they can act directly on IECs or can influence immune responses in the intestinal stroma. Examples of such metabolites are short-chain fatty acids (SCFAs) such as butyric acid, acetic acid and propionic acid, which are the main metabolic end-products of bacterially fermented undigested complex carbohydrates. SCFAs are an important energy source, not only for the gut microorganisms themselves but also for IECs, but they can also have diverse regulatory functions on host physiology and immunity and are generally described to have anti-tumorigenic effects. SCFAs have been shown to inhibit histone deacetylases (HDACs) in neutrophils, which leads to the decreased production of TNF and nitric oxide and to the inhibition of NF-κB signalling. Similar anti-inflammatory effects can be seen in macrophages, in which SCFA metabolites downregulate production of the pro-inflammatory mediators IL-6, IL-12 and nitric oxide in the gut[138]. Furthermore, SCFAs can block the generation of DCs from the bone marrow and enhance the immunosuppressive functions of FOXP3$^+$ T_{reg} cells by the inhibition of HDACs[139]. By contrast, *E. coli* stimulates HDAC3 activity in IECs through the metabolism of phytate and the production of inositol-1,4,5-trisphosphate, which promotes regeneration following intestinal damage by regulating IEC proliferation[140]. The intestinal microbiome also plays an important role in the response of CRC to therapy, particularly in the context

of immunotherapies[141]. In response to conventional chemotherapy, which triggers epithelial cell apoptosis in the ileum, ileal bacteria such as *Bacteroides fragilis* and *Erysipelotrichaceae* signal to migratory DCs to activate T follicular helper cells that colonize the tertiary lymphoid structures associated with colorectal tumours and, together with B cells, promote the anti-tumorigenic effects of chemotherapy and immunotherapy[142].

The mycobiome. The notion that changes in fungal communities and the associated consequences for host physiology and pathology are also relevant to CRC is only recently becoming more evident (FIG. 3b). So far, the focus has been on the composition of fungal communities rather than on their functional analysis. By comparing different studies on the fungal composition of the human mycobiome, a large variability has been observed, with relatively few species belonging to the genera *Aspergillus*, *Candida*, *Cladosporium*, *Cryptococcus*, *Galactomyces*, *Malassezia*, *Saccharomyces* and *Trichosporon* showing more consistent abundance[143].

Fungi can directly promote carcinogenesis through mycotoxins (for example, aflatoxins, sterigmatocystin, fumonisin B, ochratoxins A, trichothecenes and patulin) derived from pathogenic species in contaminated food, including *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp., which are often related to tumorigenesis in the gastrointestinal tract[144]. Sterigmatocystin, a late metabolite in the aflatoxin pathway, has been shown to induce intestinal metaplasia and to promote the progression of gastric carcinomas in the presence of *Helicobacter pylori*[145,146].

By contrast, the influence of commensal fungi is less clear. Several studies have looked at the association of specific intestinal fungal species with the intestinal immune system of the host[147–149]. The recognition of fungal cell wall components by pattern-recognition receptors on antigen-presenting cells activates tyrosine kinases, such as SYK and caspase-associated recruitment domain 9 (CARD9), which in turn activate inflammatory responses involving NF-κB, the production of cytokines, such as IL-17A and IL-22, and the induction of innate and adaptive immune responses. Recently, the analysis of *Card9*-deficient mice in the AOM/DSS model identified a role for fungus-induced caspase 1 activation in macrophages in restricting tumorigenesis in one study[150] and *Candida tropicalis*-dependent differentiation and activation of MDSCs in another study[151]. Although, in both cases, CARD9 deficiency led to the same net outcome — that is, an increased tumour load and more aggressive colon tumours — the underlying mechanisms were different, which highlights the high levels of complexity in this field even when analysing the same gene. More detailed studies will be necessary to better understand the functional consequences of changes in the composition of individual fungal species. It will also be important to focus on other intestinal microorganisms such as protozoa and viruses, including phages.

Conclusions and therapeutic prospects

Despite the finding that most types of CRC have only a few deregulated cell-surface markers and intracellular signalling pathways in common, such as hyperactivation of WNT–β-catenin or RAS signalling, inhibiting those markers and pathways has often not been effective for the treatment of CRC. However, we are now beginning to better understand how CRC onset, progression and treatment outcome are highly influenced by stromal and immune cells in the TME. As outlined here, inflammation affects every stage of CRC tumorigenesis and modulates the polarization of cells in the TME as well as the corresponding cytokine milieu. Combinatorial approaches targeting various immune cell types in the TME are currently being trialled (TABLE 2). Furthermore, extrinsic factors, such as environmental exposure, dietary habits, and commensal and pathogenic microbiome composition, can directly and indirectly alter the behaviour of tumour cells as well as of cells within the TME, which also affects disease progression and response to treatment. Improving our knowledge of how these factors function together to induce tumour-initiating inflammation or to promote tumour growth by secreting growth-stimulatory cytokines or by mediating T cell suppression will help to develop strategies targeting specific inflammatory pathways in the context of CRC.

Significant technological advances, such as multi-omics and single-cell analysis[152], CRISPR-mediated in vivo screens, and the development of human organs-on-a-chip, have helped in understanding this complex network of interactions. By using large-scale omics technologies, we are now very well equipped to determine the predictive biomarkers of therapeutic response[153–155] that could be used for the personalization of therapies and to reduce any adverse treatment effects.

Furthermore, huge progress has been made in the ex vivo modelling of CRC, especially with regard to effects of the TME. Bioengineered submucosal organoids with embedded CRC cells have been used to study the effects of stromal topography on the cancer cell phenotype and the response to chemotherapy[156]. Decellularized human biopsy samples recellularized with CRC cells to generate an organotypic 3D model that mimics the in vivo TME can be exploited for pharmacological testing[157]. Systems such as multi-site metastasis-on-a-chip for the assessment of the metastatic preference of CRC cells[158] or other organ-on-a-chip platforms will prove very useful to better understand the mechanisms underlying metastasis or for in vitro drug screens for metastatic CRC. An air–liquid interface method to co-culture patient-derived organoids or mouse tumour epithelia from syngeneic immunocompetent hosts together with native embedded immune cells has enabled epithelial–immune cell interactions to be studied systematically[159]. Both mouse organoids and human patient-derived organoids can be used to model the effects of immune-checkpoint blockade and of the activation of tumour antigen-specific tumour-infiltrating lymphocytes. Organoid propagation of primary or metastatic tumour epithelium together with the corresponding immune stroma should enable more comprehensive immuno-oncology investigations and facilitate and improve personalized immunotherapy testing. Additionally, tumour organoid co-cultures with peripheral blood lymphocytes as a platform to enrich tumour-reactive T cells from patient blood can

Table 2 | **Current clinical trials targeting the immune system in colorectal cancer**

ClinicalTrials.gov identifier	Clinical trial phase	Type of colorectal cancer	Immune component of trial
NCT02912559	Phase III	Stage 3 CRC with MMR deficiency	T cell activation in combination with chemotherapy
NCT03866239	Phase Ib	Stage 4 MSS metastatic CRC	T cell activation
NCT03555149	Phase Ib/II	Metastatic CRC	T cell activation, immune-checkpoint blockade, activation of innate immunity via PAMPs, CD40 antigen stimulation
NCT03058289	Phase I/II	Non-MSI^{high} and/or MMR-proficient CRC	INT230-6 is a cisplatin-based intratumoural cancer therapeutic vaccine used in combination with T cell activation (anti-PD1 and anti-CTLA4 therapy)
NCT03289962	Phase Ia/Ib	Incurable, advanced adenocarcinoma	Evaluation of RO7198457, a drug that targets up to 20 tumour-associated antigens, alone or in combination with atezolizumab (anti-PDL1)
NCT02628067	Phase II	MMR-deficient/MSI^{high} CRC	T cell activation by PD1 inhibition in unresectable, metastatic solid tumours
NCT03986606	Phase I	Advanced MSI^{high} CRC	Bifunctional MabPair combination antibody product targeting PD1 and CTLA4
NCT04044430	Phase I/II	MSS, *BRAF*^{V600E}-mutated, metastatic CRC	T cell activation, immune-checkpoint blockade (anti-PD1)
NCT04354246	Phase I	CRC that has exhausted all available therapy	T cell activation using antibodies against the immuno-oncology target TIGIT
NCT03113188	Phase I	Advanced and metastatic MSS CRC	T cell activation, immune-checkpoint blockade (anti-PDL1)
NCT04599140	Phase I/II	Metastatic or unresectable MSS, *RAS*-mutated CRC	Blockade of MDSC recruitment in combination with T cell activation (anti-PDL1)
NCT04231526	Phase II	Resectable MSI^{high} CRC	T cell activation, immune-checkpoint blockade (anti-PD1)
NCT04157985	Phase III	Advanced MMR-deficient/MSI CRC	T cell activation, immune-checkpoint blockade (anti-PD1 or anti-PDL1)
NCT04017650	Phase I/II	MSS, *BRAF*^{V600E}-mutated, unresectable or metastatic CRC	T cell activation, immune-checkpoint blockade (anti-PD1)
NCT04362839	Phase I	Metastatic, chemotherapy-resistant CRC	T cell activation, immune-checkpoint blockade (anti-PD1 or anti-CTLA4)
NCT03801915	Phase II	Metastatic CRC	T cell activation by targeting CA19-9 antigen on cancer cells
NCT03436563	Phase I/II	Metastatic or unresectable MSI CRC	T cell activation, immune-checkpoint blockade (anti-PD1) and TGFβR2 inhibition
NCT02890758	Phase I	Metastatic or advanced CRC	NK cell transfer-mediated tumour cell killing
NCT04691375	Phase I	MSI^{low} and CPI refractory MSI^{high} CRC with metastatic disease	Depletion of TREM2^{+} tumour-associated macrophages
NCT04486378	Phase II	Stage 2/3 CRC	RO7198457 has the potential to increase anti-tumour activity by increasing the number of neoantigen-specific T cells
NCT03745326	Phase I/II	Metastatic or unresectable, *KRAS*^{G12D}-mutated CRC	T cell activation using anti-KRAS-G12D monoclonal TCR^{+} cells

CRC, colorectal cancer; CPI, checkpoint inhibitor; MDSC, myeloid-derived suppressor cell; MMR, mismatch repair; MSI, microsatellite instability; MSS, microsatellite stability; NK, natural killer; PAMP, pathogen-associated molecular pattern; TCR, T cell receptor; TGFβR2, transforming growth factor-β receptor type 2.

be assessed for the killing efficiency of matched tumour organoids at the level of the individual patient[160].

However, despite these impressive technical achievements, approaches to overcome T cell exclusion from tumours have yet to be developed. Here, CRISPR-mediated in vivo screens might be very useful as they have recently discovered pathways that might be involved in the unresponsiveness of tumours to immune-checkpoint blockade[161]. In addition to this, there is an ongoing need for improved in vivo tumour models such

as humanized mice. Here, the ultimate goal is to generate patient-derived xenograft models with a fully competent human immune system derived from the same patient.

In addition to the discovery of new treatment approaches, we should also use our knowledge of the immune pathogenesis of CRC to design preventive approaches and programmes to reduce cancer risk. For example, diet and dietary supplements might have a large impact on the composition and function of the intestinal microbiome and mycobiome. The prevention of CRC through such approaches would be a much better and more economical way to fight cancer than treating already developed tumours.

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