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Review article

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The immune microenvironment of colorectal cancer

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Abstract

Colorectal cancer (CRC) progression depends on the close interaction of tumour cells and the tumour microenvironment (TME). Although the TME contributes to poor therapy responses and immune evasion, immune cells within the TME can be therapeutically leveraged, as exemplified by immune checkpoint blockade (ICB). Unfortunately, only a small subset of patients with CRC benefit from ICB therapy; those with immune-activated, microsatellite unstable CRC respond, whereas the predominant group of patients with CRC, those with microsatellite-stable tumours, do not. Although challenging, modulating the TME of CRC to convert these lowly immunogenic and immunosuppressed tumours into immune-activated tumours holds tremendous therapeutic potential. In this Review we provide an overview of the cellular and molecular components of immunity in the TME of CRCs at various stages of disease as well as the mechanisms of immunosuppression and immune evasion. We further describe how systemic and local therapies for CRC impact the tumour and systemic immune microenvironments, and how immunity could serve as a therapeutic and prognostic biomarker. Lastly, we highlight novel immunotherapeutic strategies and approaches that modulate the TME of CRCs to make them amenable to immunotherapy.

Sections

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Introduction

Colorectal cancer (CRC) is the third most frequently diagnosed cancer worldwide. The incidence, prevalence and mortality of CRC remain high in industrialized countries and are expected to rise further in the next decade, especially in patients under the age of 50 years[1]. Although 5-year-survival rates are 91% and 82% for patients with stage I and stage II disease, respectively, these figures decline to 12% for stage IV disease (Fig. 1) due to the ineffectiveness of current treatment regimens for late-stage and advanced metastatic disease, underscoring the need for more effective therapies[2]. Immune checkpoint blockade (ICB) using monoclonal antibodies has gained traction in the treatment of various cancers; most notably, ICB has revolutionized the treatment of melanoma, allowing long-term disease control in patients with metastatic disease[3]. Unfortunately, most gastrointestinal tumours are notoriously resistant to ICB[4], and only approximately 15% of patients with CRC who present with mismatch repair-deficient (dMMR) or microsatellite instability-high (MSI-H) tumours respond[5]. In these patients, the therapeutic benefit of ICB exceeds that of conventional therapies, and can stabilize metastatic disease for several years[6]. It is therefore unsurprising that ICB is poised to become the standard of care for metastatic dMMR and MSI-H CRC[6]. The remaining patients with CRC generally present with mismatch repair-proficient (pMMR) or microsatellite stable (MSS) tumours and due to their lack of response to ICB and the limited efficacy of conventional therapy, the survival of these patients with stage IV cancer is still restricted to approximately 30 months after diagnosis[7]. Whereas dMMR and MSI-H CRCs display an immune-activated, so-called 'immune hot' tumour microenvironment (TME) with infiltrating functional effector cells, most pMMR and MSS CRCs are 'immune cold', explaining their resistance towards immune interventions[8]. In addition to MMR and microsatellite status, the consensus molecular subtypes (CMSs; Fig. 1) provide a gene expression-based classification system of primary CRC that allows assignment of individual tumours to one of the four CMSs (CMS1–CMS4) that are associated with distinct molecular pathways, pathological variables such as tumour sidedness (see below) and clinical outcome[9]. Importantly, the CMS correlates with particular TMEs: whereas the pMMR and MSS CRCs of CMS2 and CMS3 lack substantial stromal and immune infiltration, CMS1 tumours are densely populated by functional effector cells and show a strong overlap with dMMR and MSI-H CRCs. Lastly, the pMMR and MSS CRCs of CMS4 are enriched for signatures of stromal and myeloid cells, cytokines and immunosuppression, and have a particularly bad prognosis when compared with tumours of the other three CMSs[9]. Given the clinical potential of immunotherapy and the relevance of the immune system to tumorigenesis, a thorough understanding of the innate, adaptive and stromal immune players that shape the TME of various subtypes of CRC will be essential for improving patient outcome.

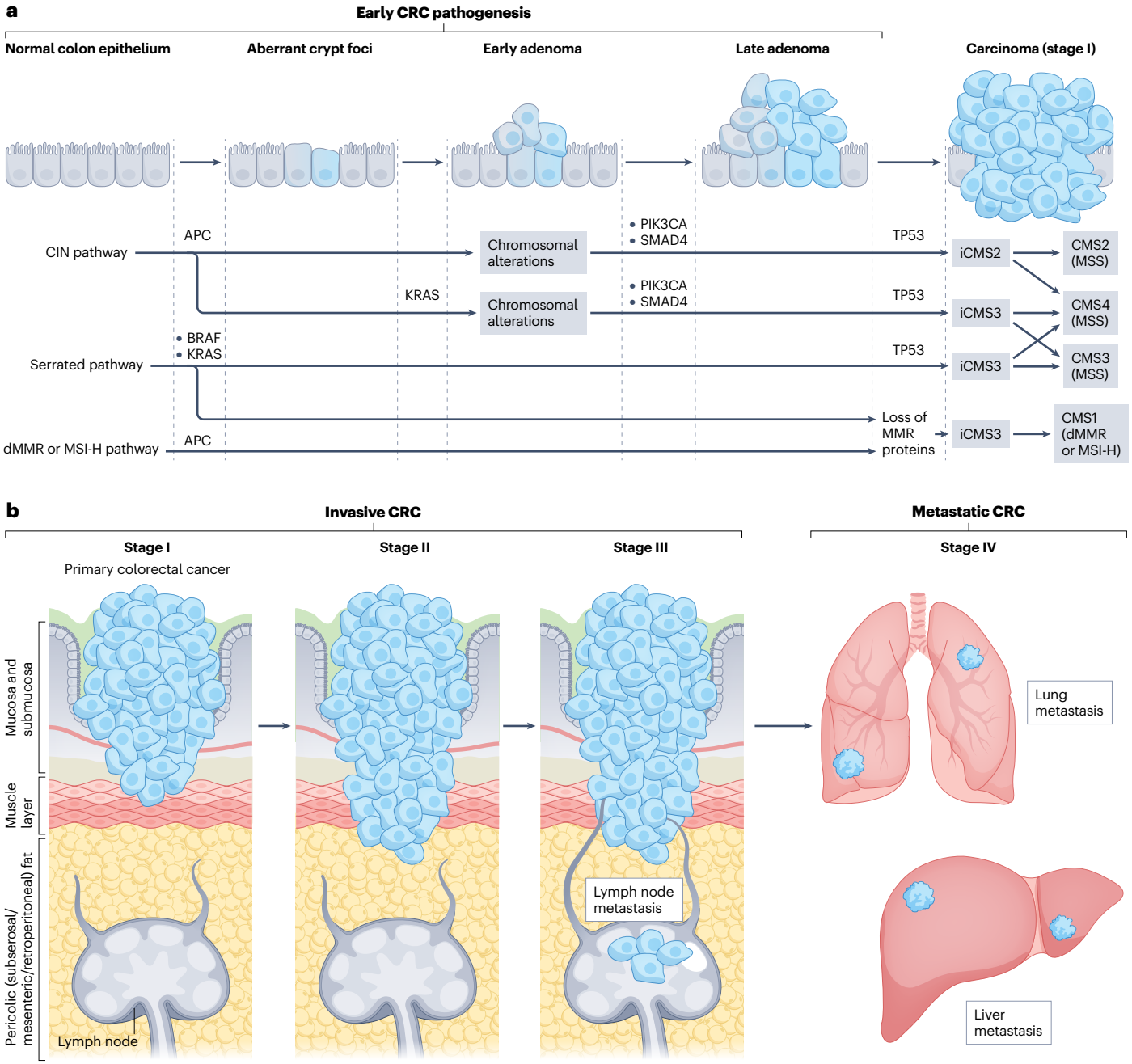
In this Review, we provide a concise overview of the cellular and molecular components of the CRC immune environment, emphasizing the phenotypic diversity and functional plasticity of the cells, as well as the role of standard systemic therapy in remodelling the immune microenvironment. Moreover, we discuss strategies for how specific immune-related features can serve as clinical biomarkers to select patients with CRC for ICB and other therapies, and outline new immunotherapeutic strategies in CRC. Please note that an in-depth discussion of both the role of the gut microbiota and the role of innate lymphoid cells in CRC pathogenesis is beyond the scope of this Review, and instead we refer readers to comprehensive recent reviews (refs. 10,11 and refs. 12,13, respectively).

The immune microenvironment of CRC

CRC pathogenesis is a stepwise process during which normal colonic epithelium transforms into benign progenitor lesions which can ultimately progress to invasive CRC. According to their macroscopic and histological appearance, the sequence of molecular events and the time from development of the first progenitor lesions to full-blown carcinoma, three pathways of CRC pathogenesis can be distinguished: the traditional or chromosomal instability (CIN) pathway (approximately 70% of cases); the serrated pathway (approximately 15% of cases); and the dMMR or MSI-H pathway (approximately 15% of cases)[14] (Fig. 1). The CIN pathway is initiated by a loss-of-function mutation of the tumour suppressor gene *APC*. Inactivating *APC* mutations lead to uncontrolled WNT/β-catenin signalling in colonic epithelial cells which promotes cellular proliferation and the formation of aberrant crypt foci, the earliest pathological lesions during CRC pathogenesis[15]. Chromosomal alterations together with further genetic or epigenetic alterations including mutations in oncogenes and tumour suppressor genes such as *KRAS*, *NRAS*, *SMAD4*, phosphatidylinositol-4,5-bisphosphate

Fig. 1 | Pathways of CRC pathogenesis, transcriptomic subtypes and clinical stages of CRC. a, Colorectal cancer (CRC) arises from colon epithelium following a histologically and molecularly heterogeneous adenoma-to-carcinoma sequence. The majority (approximately 70%) of cases follow the chromosomal instability (CIN) pathway, which can take several decades to form invasive CRC and involves the formation of classical tubular adenomas. Mutations of the *APC* gene are the initiating event of this pathway, which is followed by other genomic mutations (for example, in the *KRAS*, *PIK3CA* or *SMAD4* locus) and major chromosomal alterations including gains in chromosomes 7, 8, 13 and 20 and losses of 1, 4, 8, 14, 15, 17 and 18 (refs. 14,48). The CIN pathway usually results in invasive carcinomas that belong to the bulk transcriptomic consensus molecular subtype 2 (CMS2), CMS3 or CMS4 (37%, 13% and 23% of all CRCs, respectively[9]). A recent study further refined the CMS classification based on single-cell transcriptomic signatures of tumour cells and demonstrated that two tumour epithelial subtypes exist within the different CMS of CRC, intrinsic CMS2 (iCMS2) and intrinsic CMS3 (iCMS3). Among other factors, iCMS2 and iCMS3 differ with respect to *KRAS*, *PIK3CA* and *BRAF* mutations that predominantly occur in iCMS3 tumours[48]. Importantly, fibrotic tumours of the combined bulk CMS4 and iCMS3 subtype have a particularly bad prognosis, suggesting that a separate analysis of tumour cell and tumour microenvironment (TME) features within CRCs could be prognostically more informative than bulk-based methods[48]. Compared with tumours developing via the CIN pathway, CRCs resulting from the serrated pathway develop much faster (within a couple of years) and arise from saw-toothed (that is, serrated) histological lesions of the colorectal mucosa[14]. Mutations in the *BRAF* locus, particularly V600E mutations, are a hallmark of these serrated lesions[14]. A subset of serrated CRCs shows loss of DNA mismatch repair (MMR) proteins which results in microsatellite instability-high (MSI-H) tumours of CMS1 that respond to immune checkpoint blockade (ICB). Similarly, tumours arising via the mismatch repair-deficient (dMMR) or MSI-H pathway display genomic or epigenomic loss of MMR proteins, are MSI-H, belong to CMS1 and do not bear *BRAF* but *APC* mutations. **b**, The clinical stages of invasive and metastatic CRC (mCRC). Stage I, the tumour infiltrates the muscle layer of the colon but does not penetrate it; stage II, the tumour infiltrates the pericolic fat but has not established metastases in the pericolic lymph nodes; stage III, the tumour has formed metastases in the pericolic lymph nodes; and stage IV, the tumour has established distant metastases. The liver or the lungs are the most frequent sites of CRC metastases. APC, APC regulator of WNT signalling pathway; MSS, microsatellite stable; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit-α.

3-kinase catalytic subunit-α (*PIK3CA*) and *TP53* give rise to adenomas, which can eventually progress to invasive CRC. Intriguingly, associations of the developmental pathways and the microenvironment of resulting CRCs have been noted; for example, the CIN pathway usually results in CRC tumours that are pMMR or MSS, and are either immune-cold CMS2 or CMS3, or immunosuppressive CMS4 (ref. 14). *BRAF*-mutated serrated lesions typically result in either pMMR or MSS CMS3 tumours or immune-activated dMMR or MSI-H tumours of the CMS1 subtype that respond to ICB[14] (Fig. 1). Lastly, these developmental pathways and mutational patterns are associated with the anatomical location of the resulting primary tumour. For example, *BRAF*-mutant, CMS1 CRCs arise more frequently in the right colon whereas *KRAS*-wild type, CMS2 tumours are more often found in the left colon and rectum[9]. Although the exact biological mechanisms responsible for these clinical observations remain unknown, it is speculated that the different embryonic origins of the left and right colon are relevant[16]. Additionally, right and left-sided tumours also differ regarding microbiome composition and their immune microenvironment[17]. In the following sections, we outline how the colorectal immune microenvironment affects the various steps of CRC pathogenesis and how it is shaped by environmental factors including therapy.



The immune microenvironment during early CRC pathogenesis

The colon mucosa is exposed to various nutrients and toxicants, as well as the gut microbiome, which together set a state of controlled inflammation[18]. Consequently, disturbances of this delicate balance can result in uncontrolled inflammation, which in turn fosters the growth and invasive potential of adenomas through cytokine-driven and inflammation-driven mutagenesis and immunosuppression[19] (Fig. 2). These same mechanisms can also be found in established CRCs (Fig. 3). The impact of inflammation on CRC development is strikingly illustrated by ulcerative colitis, an inflammatory bowel disease which leads to a relapsing–remitting inflammation of the colon mucosa and, consequently, increases the risk of CRC in affected individuals[20]. Mouse models mimicking colitis-associated tumorigenesis, such as the azoxymethane/dextran sulfate sodium (AOM/DSS) model, have been invaluable for deciphering mechanisms of early CRC development[21–27]. Mechanistically, inflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-11, IL-17, IL-22 and tumour necrosis factor (TNF) secreted by myeloid cells, T cells and fibroblasts or bacteria-derived lipopolysaccharide (LPS) activate nuclear factor-κB (NF-κB), signal transducer and activator of transcription 3 (STAT3) and other signalling pathways in colonocytes, nurturing the proliferative potential of transformed colonocytes[21–26,28–32] (Fig. 2). Furthermore, reactive oxygen and nitrogen species (RONS) that are generated by infiltrating myeloid cells in response to pro-inflammatory stimuli, such as LPS and inflammatory cytokines, give rise to DNA damage and mutations in epithelial cells, linking inflammation and mutagenesis[33,34]. Moreover, peroxynitrite ($ONOO^-$), a RONS produced by myeloid-derived suppressor cells (MDSCs), potently inhibits T cell, B cell and natural killer cell responses, thereby prompting immunosuppression[35]. MDSCs are recruited to the inflamed colon mucosa via the C–X–C motif chemokine ligand 1 (CXCL1) where they inhibit $CD8^+$ T cell cytotoxicity[36]. Recruitment of immunosuppressive myeloid cells is also driven by mutations in CRC driver genes such as *KRAS* and *SMAD4*, which lead to increased secretion of CXCL3 and CXCL1, respectively[37,38]. Conversely, genetic loss and pharmacologic inhibition of the CXCL1 and CXCL3 receptor, CXCR2, inhibits both inflammation-associated and sporadic CRC in mice[36,39].

Notably, most findings from inflammation-associated models are also relevant for sporadic tumorigenesis, underscoring the fundamental importance of inflammation for CRC growth. For example, mutations in CRC driver genes such as *TP53* or *APC* compromise the intestinal barrier and lead to bacterial invasion and elicitation of a pro-tumoural inflammatory microenvironment in adenomas which is marked by activation of NF-κB and STAT3 pathways in epithelial cells[40,41]. In mice, activation of NF-κB in *TP53*-deficient epithelial cells occurs through both epithelial cell-intrinsic mechanisms and through extrinsic stimulation by inflammatory myeloid cells, and this in turn enhances epithelial-to-mesenchymal transition, thereby aggravating invasion and metastasis of colorectal adenomas[40]. Single-cell RNA sequencing and spatial transcriptomics have begun to confirm findings from preclinical studies[42–46]. For example, patient aberrant crypt foci show upregulation of gene signatures associated with NF-κB activation in epithelial cells and an increased presence of $CD8^+$ T cells expressing the exhaustion marker PD1 compared with normal colon mucosa[43,44]. Moreover, single-cell RNA sequencing of human normal mucosa, colorectal adenomas and carcinomas demonstrated a continuous increase across disease stages in the percentage of regulatory T cells (T_{reg} cells) and activated fibroblasts[45], indicators of immunosuppression in CRC. It is interesting to note that human inflammatory bowel disease-associated CRCs more frequently exhibit the stroma-rich CMS4 compared with sporadic CRCs, suggesting that excessive mucosal inflammation promotes the development of an immunosuppressive fibrotic reaction[47]. In accordance, it was recently suggested that the fibrotic component of CMS4 tumours develops independently of tumour-intrinsic genetic or transcriptomic alterations[48] and it is tempting to speculate that the high concentrations of pro-inflammatory and fibrotic cytokines such as IL-1, IL-6 family members and transforming growth factor-β (TGFβ) observed during active and resolving mucosal inflammation are needed for the generation of these prognostically detrimental tumours. For example, colitis promotes a pro-tumorigenic IL-11-expressing inflammatory fibroblast population in both humans and mice[49,50] and IL-11 is key for fibrogenesis in CRC[51]. Additionally, high concentrations of TGFβ, which stimulates proliferation and pro-fibrotic pathways in fibroblasts, is a signature of CMS4 tumours[9] and can be observed in mucosal samples from patients with active ulcerative colitis[52]. Although a very small proportion of CMS4 CRCs develops in patients with ulcerative colitis[53], these findings highlight the importance of inflammation-associated pathways for fibrogenesis in CRC and suggest that extrinsic pro-inflammatory factors could be a cause of CMS4 CRC.

Dietary habits and alcohol consumption are extrinsic factors that influence the colonic immune microenvironment and can promote inflammation[54]. A 'western diet', characterized by a diet high in fat and carbohydrates and low in fibres, along with a lifestyle of physical inactivity, alcohol consumption and tobacco use are well-known risk factors for the development of CRC[15]. In mice, a high-fat diet inhibits dendritic cell responses, leading to gut dysbiosis and *KRAS*-dependent intestinal tumour growth[55]. Intriguingly, high-fat diet-induced obesity in mice promotes inflammation in the colon mucosa[56], suggesting that the increased risk for CRC development in individuals with obesity may be inflammation-driven. Conversely, physical exercise reduces the risk of developing CRC in both mice and humans[57], and a general, systemic decrease in inflammatory parameters and a reduction of the pro-tumorigenic inflammatory lipid mediator prostaglandin E_2 (PGE_2) in the colon mucosa are observed in physically active individuals[57,58]. This suggests that an alleviation of inflammation might be relevant for the protective effect of physical activity on the risk of developing CRC. Although mechanistic evidence supporting the role of alcohol in CRC pathogenesis is scarce, it was demonstrated that ethanol exposure aggravates tumour growth in the AOM/DSS mouse model and is associated with the upregulation of IL-1, IL-6 and TNF in colonic mucosa[59,60]. Furthermore, ethanol consumption is associated with systemic dysfunction of natural killer cells and T cells in both humans and mice, which could affect immunosurveillance during early-stage CRC pathogenesis[60].

Finally, in addition to the pro-proliferative and immunosuppressive effects of inflammatory cytokines, colonocytes themselves acquire traits that promote immune evasion. For example, loss of STAT3 in colonocytes augments major histocompatibility complex (MHC) class I-dependent antigen presentation and, thus, $CD8^+$ T cell activation through a complex mechanism involving increased mitophagy, lysosomal membrane permeability and antigen processing[61]. Conversely, mitophagy defects in tumour cells have been shown to constitute a tumour-promoting mechanism in CRC and other cancers[62,63]. Furthermore, SRY-box transcription factor 17 (SOX17), an embryonal transcription factor, is activated in early-stage colorectal tumour cells and leads to downregulation of the interferon-γ (IFNγ) receptor, resulting in diminished expression of MHC class I and reduced $CD8^+$

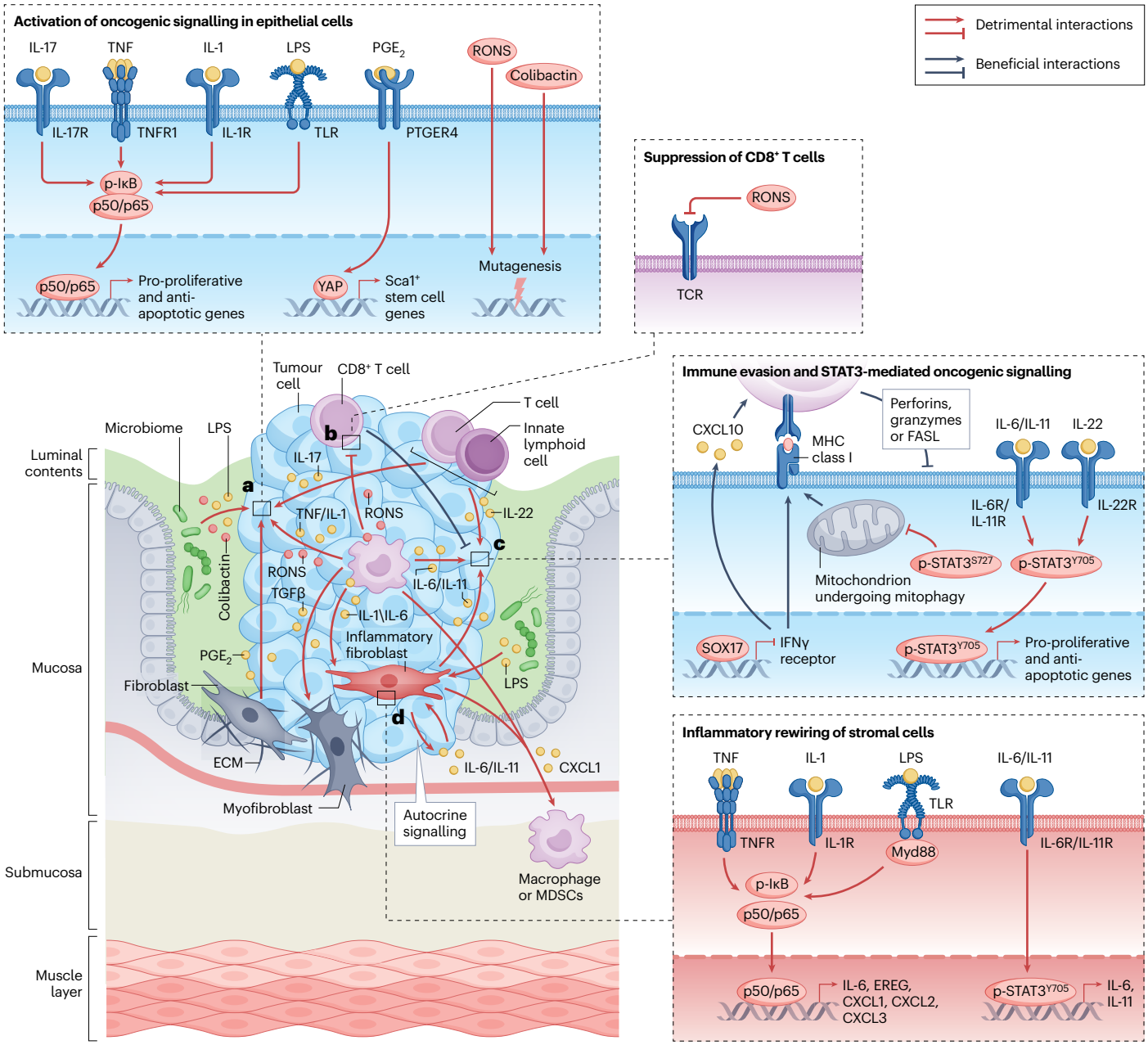


Fig. 2 | Mechanisms of inflammation-driven tumorigenesis, immunosuppression and immune evasion during early CRC pathogenesis. a, During the early pathogenesis of colorectal cancer (CRC), pro-inflammatory cytokines, bacterial products (for example, lipopolysaccharide (LPS)) and lipid mediators (for example, prostaglandin E_2 (PGE_2)) foster epithelial cell proliferation and stemness. Reactive oxygen and nitrogen species (RONS) produced by inflammatory cells along with bacterial toxins such as colibactin[233] trigger mutations in epithelial cells that drive tumour growth. **b**, RONS secreted by infiltrating myeloid-derived suppressor cells (MDSCs) inhibit T cell responses, for example through peroxynitrite ($ONOO^-$)-mediated nitration of the T cell receptor (TCR)[234]. **c**, Oncogenic signalling, for example, via SRY-box transcription factor 17 (SOX17), helps epithelial cells evade $CD8^+$ T cell-mediated killing. Also, signal transducer and activator of transcription 3 (STAT3) activation by interleukin-6 (IL-6), IL-11 or IL-22 supports tumour cell proliferation and survival[23,24] as well as mitochondrial integrity, which can impact antigen presentation by tumour cells[61]. **d**, Inflammatory fibroblasts stimulated via tumour necrosis factor (TNF), IL-1 (ref. 235), LPS[236], IL-6 or IL-11 (ref. 237) amplify inflammatory reactions through the production of cytokines and chemokines. Transforming growth factor-β (TGFβ)-induced activation of myofibroblasts during resolution of inflammation triggers secretion of extracellular matrix (ECM) factors. Both heightened inflammatory cytokine production as well as ECM deposition are hallmarks of consensus molecular subtype 4 (CMS4) carcinomas, which suggests that inflammatory fibroblasts may be involved in the generation of CMS4 tumours during early CRC pathogenesis. CXCL1, C–X–C motif chemokine ligand 1; EREG, epiregulin; FASL, Fas ligand; IFNγ, interferon-γ; IL-1R, IL-1 receptor; MHC, major histocompatibility complex; Myd88, MYD88 innate immune signal transduction adaptor; NF-κB, nuclear factor-κB; p-IκB, phosphorylated NF-κB inhibitor; PTGER4, prostaglandin E receptor 4; TLR, Toll-like receptor; TNFR, TNF receptor; YAP, yes1-associated transcriptional regulator.

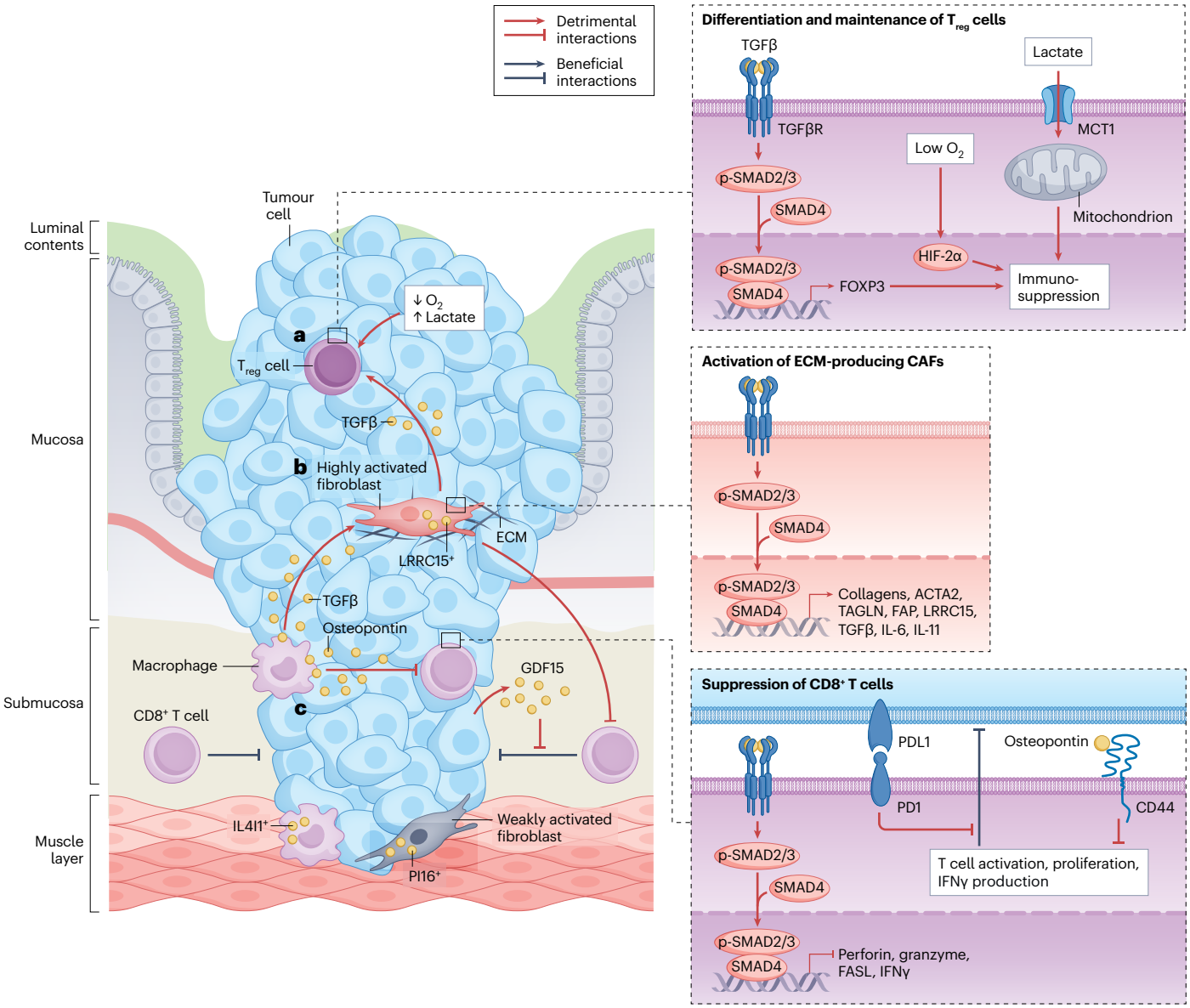


Fig. 3 | Immunosuppressive mechanisms in invasive CRCs. In invasive colorectal cancer (CRC), immunosuppression is mediated through accumulation of regulatory T cells (T_{reg} cells), immunosuppressive macrophages and highly activated fibroblasts that secrete transforming growth factor-β (TGFβ) and osteopontin. **a**, TGFβ is a key immunosuppressive cytokine inducing T_{reg} cells[79], the immunosuppressive capacity of which is also dependent on local oxygen and nutrient availability[98]. **b**, TGFβ is further responsible for activating a myofibroblastic phenotype in cancer-associated fibroblasts (CAFs), leading to the secretion of extracellular matrix (ECM) proteins, interleukin-6 (IL-6) and IL-11 (ref. 95). **c**, Osteopontin and TGFβ along with PDL1 can also act directly on $CD8^+$ T cells to suppress their anti-tumorigenic activity. GDF15 has emerged as another immunosuppressive cytokine secreted by CRC cells to promote immune exclusion and resistance to immune checkpoint blockade (ICB)[238,239]. Notably, TGFβ, osteopontin, IL-6 and IL-11 are mainly secreted by cells in the CRC microenvironment of the inflamed and microsatellite instability-high (MSI-H) consensus molecular subtype 1 (CMS1) and mesenchymal and microsatellite stable (MSS) CMS4 subtypes[9,103], suggesting that these immunosuppressive and pro-inflammatory cytokines are particularly relevant here. In contrast, the MSS CMS2 and CMS3 subtypes are generally less infiltrated with immune cells, particularly T cells[240,241], and therefore targeting pro-inflammatory and/or immunosuppressive signalling cascades might be less effective in these tumours. FAP, fibroblast activation protein; FASL, Fas ligand; FOXP3, forkhead box P3; HIF-2α, hypoxia-inducible transcription factor 2α; IFNγ, interferon-γ; LRRC15, leucine rich repeat containing 15; MCT1, monocarboxylate transporter 1; TAGLN, transgelin; TGFβR, TGFβ receptor.

T cell-dependent killing of $LGR5^-$ stem cells[64]. Importantly, the capacity of colorectal stem cells to switch from an $LGR5^+$ to $LGR5^-$ state and induce a so-called 'fetal' programme seems to be an important feature for tumour progression and resistance to both conventional therapies and the patient's immune system[65]. Moreover, cyclooxygenase 2 (COX2)-expressing fibroblasts in the lamina propria induce the

expansion of a yes1-associated protein (YAP)-dependent, Sca1$^+$ stem cell population in colon adenomas through the production of PGE_2, thereby augmenting colonic tumour growth[66]. Importantly, YAP activation in cancer cells drives several immunosuppressive mechanisms including the upregulation of the immune checkpoint PDL1 and the production of molecules that recruit MDSCs[67]. In addition, PGE_2 production also limits the expansion of CD8$^+$ T cells in a mouse model of CRC, supporting that PGE_2 is a pro-tumorigenic factor during CRC development[68]. Indeed, pharmacologic blockade of COX2 is effective as chemoprevention in patients with colorectal adenomas, but has cardiovascular and gastrointestinal side effects that complicate prolonged application[69]. Interestingly, recent retrospective analyses suggest that COX2 inhibition might be beneficial for the adjuvant treatment of patients with stage III CRC harbouring PIK3CA mutations[70]. It may be that short-term application of these drugs in defined settings is an option, but further studies are necessary.

The immune microenvironment of invasive CRC

As colorectal lesions evolve into CRC, the surrounding microenvironment supports tumour growth and promotes immune evasion (Fig. 3). At the invasive front of CRCs, tumour cells are in direct contact with normal tissue, which can provoke an inflammatory response. As discussed in more detail later, the extent of immune cell infiltration and, specifically, the number of T cells at the invasive front strongly correlate with patient survival and may also have therapeutic implications[71,72]. In line with this, the formation of tumour buds at the invasive front, a histological hallmark of aggressive CRC, is associated with a less dense immune infiltrate and worse prognosis, further supporting that immunosuppression is a crucial feature of invasive CRCs[73]. Intriguingly, a recent study demonstrated that T_{reg} cells, which limit effector T cell functions and dampen the antitumour response[74], are the most prominent immune cells adjacent to tumour buds at the invasive front of CRCs[75]. T_{reg} cells inhibit CD8$^+$ T cells in CRC (for example, via the checkpoint molecule CTLA4)[76]. Another immune checkpoint molecule, PDL1, is highly expressed on myeloid cells at the invasive margin, hinting towards the PD1–PDL1 axis being important for myeloid-driven T cell suppression at the invasive margin[75]. Intriguingly, tumour-associated macrophages also express PD1 and PD1–PDL1 ligation blocks phagocytosis of tumour cells by tumour-associated macrophages in mouse models of CRC[77], all suggesting that PD1–PDL1 interactions are important for immunosuppression at the invasive margin of CRCs.

An important factor that drives functional heterogeneity of immune and stromal cells in the TME of CRC is TGFβ[51,78]. TGFβ is a pleiotropic immunosuppressive cytokine produced by various cell types in the TME including tumour cells, myeloid cells and fibroblasts. It is one of the major stimuli that leads to differentiation of T_{reg} cells[79]. Accordingly, in a mouse model of primary CMS4 CRC, TGFβ2-producing tumour cells activated tumour-associated neutrophils which, in turn, inhibited T cell activity, fostering metastasis[80]. Additionally, in a mouse model of CRC, the loss of TGFβ1 receptor on CD8$^+$ T cells led to improved immune cell trafficking into tumours via increased expression of CXCR3 on CD8$^+$ T cells[81]. Also, TGFβ is among the most potent stimuli giving rise to myofibroblastic cancer-associated fibroblasts (CAFs) that secrete ECM constituents such as collagens that can lead to T cell exclusion from tumours[82]. Although TGFβ inhibition stunted tumour growth and synergized with ICB in mouse models of CRC[83–87], clinical studies evaluating its potential together with ICB for the treatment of CRC and other types of cancer largely gave negative results[87], suggesting that other pathways might compensate for the loss of TGFβ signalling in the TME.

In accordance with this, hypoxia and metabolic alterations are two common phenomena of invasive tumours effecting immunity in solid tumours including CRC[88,89]. In both hypoxic tumour and TME cells, hypoxia-inducible transcription factors (HIFs) are stabilized and translocate to the nucleus where they initiate transcriptional programmes to adapt cellular metabolism and signalling to the low oxygen environment[90]. Importantly, HIFs have direct effects on immune cell functions in the TME. For example, HIF-2α is necessary for the regulation of T_{reg} cell function and loss of HIF-2α in forkhead box P3 (FOXP3)-positive T_{reg} cells slowed tumour growth in a mouse model of CRC[91]. In brain tumours, hypoxia promotes apoptosis of γδ T cells[92] and this may be relevant for MSI-H CRCs, where γδ T cells are critical for killing tumour cells with a loss of MHC expression[93]. Additionally, angiogenesis is also upregulated in response to hypoxia. Pro-angiogenic cytokines such as vascular endothelial growth factor (VEGF) or osteopontin, secreted by hypoxic tumour cells, myeloid cells and CAFs, activate endothelial cells to proliferate, migrate and form the blood vessels needed for tumour expansion in CRC[94,95]. Consequently, in patients with CRC, both high expression of VEGF and blood vessel density in the primary tumour are associated with a worse outcome[96]. Hypoxia also leads to metabolic reprogramming in favour of anaerobic glycolysis which can be exploited by tumour cells for immunosuppression[97]. For example, lactate, the by-product of anaerobic glycolysis, has broad tumour-promoting and immunosuppressive effects on CAFs, T cells and myeloid cells in in vitro and mouse models of CRC[98–100].

Lastly, it is becoming evident that the immune microenvironment of invasive CRC is spatially organized. For example, IL4I1$^+$ macrophages that express a gene signature of phagocytosis were found to be located at the invasive margin of invasive CRC and their presence correlated with an improved patient prognosis[101]. Conversely, a subset of secreted phosphoprotein 1-positive (SPP1$^+$) macrophages, known to suppress T cell activity[102], are associated with negative prognosis in patients[101,103–105] and were found preferentially located in hypoxic and necrotic regions of the tumour core[101]. In addition to hypoxia[106], several other pro-inflammatory stimuli such as IL-6 secreted by enteric glia cells can polarize monocytes to the pro-tumorigenic SPP1$^+$ phenotype in CRC[107,108] (see Box 1 for more information on the role of the nervous system in the pathogenesis of CRC). Furthermore, LRRC15$^+$ CAFs, a highly activated myofibroblast population associated with immune exclusion, immunosuppression and a detrimental outcome in various cancers[109], co-localize with SPP1$^+$ macrophages and osteopontin, the *SPP1*-encoded protein, in the tumour core[110] (Fig. 3). Conversely, PI16$^+$ and COL15A1$^+$ steady-state fibroblasts tend to localize to the invasive front where they are thought to exert antitumour functions, such as recruiting T cells via CXCL12 (ref. 110). It is conceivable that the presence or phenotypes of certain microenvironmental cell populations in defined spatial niches can serve as therapeutic biomarkers and future studies will help evaluate the clinical potential of such an approach.

The immune microenvironment of colorectal liver metastasis

Invasive CRCs can form distant metastases in the liver, lungs and peritoneum, but only rarely metastasize to the brain or bone[111]. The liver is the main organ of metastasis for CRC, such that approximately 70% of patients with metastatic CRC (mCRC) have colorectal liver metastases (CRLIMs)[111–113]. Given that surgical resection of CRLIM is routinely performed in patients[7], resulting in ample amounts of human material, it is the most well-studied CRC metastasis. For this reason, we will mainly focus on the immune microenvironment of CRLIM in this section (Fig. 4).

Box 1 | Nerves in the microenvironment of colorectal cancer

The colon is highly innervated by the autonomous nervous system, and interactions of neurons and glia cells with the tumour cells and immune, stromal and microbial compartments are increasingly recognized as important contributors to colorectal cancer (CRC) pathogenesis and therapy (reviewed in detail elsewhere[250]). For example, acetylcholine (ACh) released from cholinergic neurons activates M_3 muscarinic ACh receptors on colonic epithelial cells, which in turn leads to epidermal growth factor receptor (EGFR)–ERK–AKT signalling and promotes tumour growth in the azoxymethane/dextran sulfate sodium (AOM/DSS) model[251,252]. Additionally, serotonin produced by enteric neurons supports the self-renewal of CRC stem cells, and blockade of serotonergic signalling stunted CRC growth in the AOM/DSS model[253]. Moreover, adrenergic signalling via β-adrenergic receptors on T cells or cancer-associated fibroblasts (CAFs) seems to have immunosuppressive and growth-promoting effects in CRC. For example, exhausted T cells from human and mouse CRCs show high expression of the *ADRB1* gene (encoding the β_1-adrenoceptor)[254], and activation of β_2-adrenoceptors on CAFs increases the secretion of nerve growth factor (NGF) which reinforces tumoural adrenergic innervation and supports CRC progression via increased yes1-associated protein (YAP) signalling[255]. Taken together, the nervous system exhibits tumour-promoting functions in CRC and surgical denervation might represent a strategy for CRC treatment[256,257] but future studies are warranted.

When CRC cells metastasize to the liver, they encounter parenchymal and stromal cells, such as hepatocytes, liver sinusoidal endothelial cells and hepatic stellate cells, as well as specific types of tissue-resident innate immune cells which can act as a first line of defence. For instance, resident hepatic macrophages (also known as Kupffer cells) phagocytose incoming tumour cells and, consequently, depleting these cells increases the liver metastatic burden in an intravenous injection model of CRLIM[114]. Intriguingly, human circulating colorectal tumour cells exhibit pronounced expression of CD47, a checkpoint molecule that interacts with signal regulatory protein-α (SIRPα) on myeloid cells to inhibit phagocytosis, suggesting that metastasizing CRC cells may use this as a mechanism to escape phagocytes in the metastatic host organ[115]. Although not yet shown in CRLIM, pro-inflammatory neutrophils are also capable of eliminating tumour cells in the liver through reactive oxygen species (ROS)-dependent tumour cell killing, as demonstrated in a mouse model of hepatocellular carcinoma[116]. In contrast, neutrophil depletion or inhibition of neutrophil-intrinsic TGFβ signalling reduced the liver metastatic burden in a model of stroma-rich CRC[80], which argues for the existence of anti-inflammatory, pro-metastatic neutrophils instructed by TGFβ[117].

The acute-phase response (APR) is mounted by hepatocytes in response to systemic inflammation and circulating pro-inflammatory cytokines, such as IL-1 β, IL-6 and TNF, and leads to synthesis of acute-phase proteins such as C-reactive protein (CRP), serum amyloid A (SAA), complement factors and fibrinogen. Together these hepatic proteins strengthen the humoral defence against circulating pathogens (for example, by opsonizing bacteria[118]), but also have an impact on the hepatic microenvironment[119]. Patients with CRC regularly display activation of the APR — as indicated, for example, by elevated levels of blood CRP — and high APR markers correlate with worse survival[120]. Despite this association, mechanistic data for how this may support tumour growth in CRC are scarce. Using a mouse model of pancreatic cancer, one study demonstrated that SAA proteins are instrumental in forming a pro-metastatic niche[121]. In this study, genetic loss of SAA led to decreased metastatic burden, in part through a reduction of neutrophil infiltration into the liver and attenuation of fibronectin expression which was further confirmed in an orthotopic mouse model of CRC[121]. In contrast, recent data from an in vivo CRISPR screen in hepatocytes suggests that several APR proteins including SAA, amyloid precursor protein and caeruloplasmin protect against metastatic colonization, possibly by attracting macrophages and neutrophils[122]. In the latter study, splenic injection of tumour organoids was used to generate liver metastasis in the absence of an orthotopic primary tumour (Table 1), and thus the lack of primary tumour-induced hepatic preconditioning may explain these seemingly contradictory results.

Furthermore, the liver may exert niche-dependent effects on the overall immune composition of CRLIM as studies report an enrichment of immunosuppressive myeloid cells including neutrophils and $SPP1^+$ macrophages and depletion of B cells in CRLIM in comparison with primary tumours[123,124]. The myeloid cell-driven immunosuppression in the liver is not only relevant for CRLIM growth but also has systemic implications and hampers ICB treatment of mCRC. For example, the frequency of Fas ligand-positive ($FasL^+$) monocyte-derived macrophages is increased in CRLIM-bearing mice compared with those without CRLIM, and this macrophage population eliminates circulating antigen-specific Fas^+CD8^+ T cells, thereby creating a systemic immune desert and blunting the efficacy of ICB[125]. The association of the presence of CRLIM and a decreased efficacy of ICB also extends to the human situation: compared with patients with only colorectal lung metastases, the presence of CRLIM is associated with worse progression-free survival in response to ICB in both patients with mCRC with pMMR or MSS and in patients with mCRC with dMMR or MSI-H[126,127]. However, in patients with CRLIM, the presence of serpin family B member 2-positive ($SERPINB2^+$) macrophages in CRLIM correlates with improved survival[128], suggesting that myeloid cells with antitumour properties exist in CRLIM.

Given its function as the most metabolically active organ, the liver is particularly sensitive to damage caused by dietary habits, diabetes and obesity, which can result in steatosis and liver dysfunction[129]. Intriguingly, fat-laden hepatocytes foster an immunosuppressive environment in CRLIM, are reprogrammed by tumour cells to sustain tumour growth and can increase the side effects associated with chemotherapy[130–134]. Excessive alcohol consumption is another risk factor for the development of steatosis[135] and prolonged ethanol intake has also been associated with an increase of the pro-inflammatory cytokines TNF, IL-1β, IL-6 and IFNγ in liver tissue, decreased natural killer cell and $CD8^+$ T cell counts in peripheral blood and increased hepatic tumour load in a mouse model of CRLIM[136].

The immune microenvironment of colorectal lung metastases is understudied, in part due to the relatively low frequency of lung metastases in patients with CRC. However, preclinical studies focused on colorectal lung metastases are rare despite the existence of suitable mouse models (Table 1). Intriguingly, both MSS and MSI-H CRC lung metastases have been reported to better respond to ICB than CRLIM, suggesting that the immune microenvironment of lung and liver metastases might differ[127,137]. Data from patients with melanoma

and mouse models of pancreatic cancer suggest that lung metastases may have higher T cell and macrophage infiltration compared with liver metastases[138,139]. Deeper analyses of the immune microenvironment of colorectal lung metastases are warranted as they might yield new mechanistic insights into antitumour immune responses that could be therapeutically exploited. Conversely, in the peritoneum, CRC metastases have an unfavourable immune microenvironment with reduction of several adaptive immune cell subtypes including $CD4^+$ T helper cells and $CD8^+$ T cells, and strongly associate with CMS4 of both the metastatic lesion and the matched primary tumour. This suggests an increased capacity of CMS4 CRC to metastasize to the peritoneum along with an expansion of immunosuppressive myofibroblastic CAFs and myeloid cells in peritoneal metastases (recently reviewed elsewhere[140]). Intriguingly, a recent preclinical study demonstrated that intraperitoneal application of an anti-PD1 antibody in an organoid-based mouse model of MSS CRC reactivated T cells in peritoneal metastases, but not in liver metastases, suggesting that in spite of their immunosuppressive profile, MSS peritoneal metastases might be amenable to ICB therapy[141].

Treatment-induced remodelling of the colorectal immune microenvironment

Systemic chemotherapeutics and/or targeted therapies impact the systemic immune system (Fig. 5a) and the tumour immune microenvironment (Fig. 5b,c). For example, when lymph node metastasis is detected after surgical resection of primary CRCs (that is, stage III) or other risk factors exist, patients often receive postoperative (or adjuvant) chemotherapy to reduce the likelihood of distant recurrence[142]. Chemotherapeutics commonly used for adjuvant CRC therapy such as 5-fluorouracil and oxaliplatin are intended to kill any tumour cells remaining after surgery, but also lead to systemic immunosuppression by disrupting leukocyte development in the bone marrow, thereby influencing systemic immunity and potentially hampering treatment effectiveness[143]. Furthermore, administration of these chemotherapeutics results in

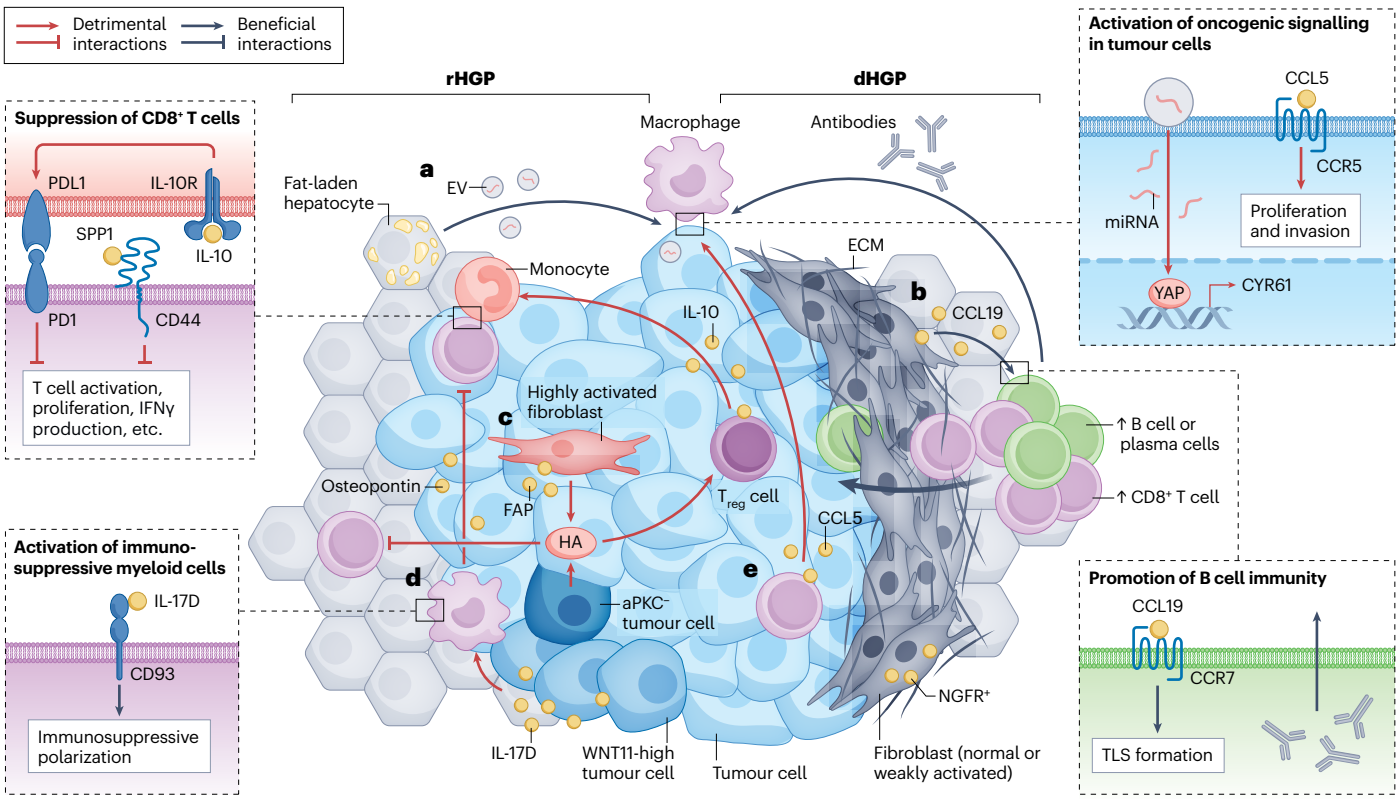


Fig. 4 | Mechanisms of immunity-mediated tumour promotion, immunosuppression and antitumour immunity in colorectal liver metastasis. a, In colorectal liver metastasis (CRLIM), colorectal cancer (CRC) cells interact with liver resident cells, for example fat-laden hepatocytes, which can transfer pro-tumorigenic microRNAs (miRNAs) through extracellular vesicles (EVs) to the cancer cells to promote growth[134]. In CRLIM with a replacement histological growth pattern (rHGP), cancer cells are in direct contact with hepatocytes. **b**, In contrast, in CRLIM with a desmoplastic histological growth pattern (dHGP), a dense fibrotic rim exists between tumour cells and hepatocytes, and is associated with T cell and B cell infiltration, and thus a better prognosis compared with a rHGP[157]. In desmoplastic CRLIM, cancer-associated fibroblasts (CAFs) at the tumour–liver interface show expression of the hepatic stellate cell marker nerve growth factor receptor (NGFR), suggesting a less activated phenotype. These CAFs also secrete C–C motif chemokine ligand 19 (CCL19), which attracts B cells and aids in the formation of tertiary lymphoid structures (TLSs) that produce antibodies to promote antitumour immunity[176]. **c**, Intratumoural CAFs in both subtypes of HGPs express fibroblast activation protein (FAP)[158], which together with atypical protein kinase C (aPKC)-negative tumour cells secrete pro-tumorigenic extracellular matrix (ECM) molecules including hyaluronic acid (HA) that promotes T cell exclusion and expansion of regulatory T cells (T_{reg} cells)[242,243]. Interleukin-10 (IL-10) secreted by T_{reg} cells stimulates immunosuppressive PDL1 expression in monocytes and, thus, immune evasion[244]. **d**, IL-17D secreted by WNT11-high tumour cells promotes an immunosuppressive phenotype in CRLIM macrophages via CD93 signalling[245]. **e**, T cell-derived CCL5 can foster tumour cell proliferation and invasion via CCR5 on tumour cells[246]. CCR5, C–C motif chemokine receptor 5; CYR61, cysteine-rich angiogenic inducer 61; IFNγ, interferon-γ; IL-10R, IL-10 receptor; SPP1, secreted phosphoprotein 1; YAP, yes1-associated transcriptional regulator.

Table 1 | Mouse models to study CRC progression

Mouse model	Type of CRC studied	Primary tumour location	Invasion of primary tumour	Metastasis	Advantages	Disadvantages	Example refs.
Chemically induced models							
AOM/DSS	Inflammation-associated CRC	Distal colon	Rarely	No	Easy to induce	High variability due to DSS Long latency (12–20 weeks) Tumour incidence depends on background strain	27
AOM	Sporadic CRC	Distal colon	Rarely	No	Easy to induce	Long latency (20–30 weeks) Tumour incidence depends on background strain but is generally low	27,214
AOM in *Tp53*$^{\Delta IEC}$ or *Tp53*$^{\Delta IEC}$/Akt1^{E17K}	Sporadic CRC	Colon	Yes	Lymph nodes, liver	Easy to induce	Requires breeding of genetically modified mice	27,40,215
Genetically modified mouse models							
Apc$^{Min/+}$	*APC*-mutated intestinal tumours	Small intestine Colon	No	No	Fast development of adenomas	No invasive cancers; adenomas are mostly in the small intestine Requires breeding of genetically modified mice	216
Apc$^{Min/+}$; *Tp53*$^{-/-}$	*APC* and *TP53*-mutated intestinal tumours	Small intestine Colon	Yes	No	Fast development of adenomas and invasive carcinomas	Requires breeding of genetically modified mice	217
Cdx2-Cre; *Apc*$^{lox/lox}$	*APC*-mutated CRC	Colon	Yes	No	Fast development of invasive carcinomas	Requires breeding of genetically modified mice	218
Apc$^{lox/lox}$;*Trp53*$^{lox/lox}$; Tet-O-LSL-Kras^{G12D}; VillinCreERT2 (so-called 'iKAP')	*APC* and *TP53*-mutated and *Kras*^{G12D}-expressing sporadic CRC	Colon	Yes	Lymph node Liver Lungs	KRAS^{G12D} can be eliminated from tumours through removal of doxycycline	Requires breeding of genetically modified mice Requires continuous administration of the antibiotic doxycycline for KRAS^{G12D} expression, altering the gut microbiome	219
Kras$^{G12D/+}$; *Trp53*$^{fl/fl}$; *Rosa26*$^{N1icd/+}$; VillinCreER (so-called 'KPN')	KRAS^{G12D}-expressing, *TP53*-mutant, Notch1-hyperactivated CRC	Colon	Yes	Lymph nodes Liver Lungs	Models development of MSS CRC via the serrated pathway	Requires breeding of genetically modified mice	80
Braf$^{LSL-V600E/+}$; *Alk5*$^{fl/fl}$; VillinCreER (so-called 'BA')	BRAF^{V600E}-mutated and TGFβ pathway-inactivated CRC	Proximal (right) colon	Yes	No	Model for right-sided CRC	Requires breeding of genetically modified mice	220
Injection of exogenous material							
Subcutaneous injection of cells, organoids or PDXs	Sporadic CRC	Subcutaneous tissue	Yes depending on the injected material	Lungs Dependent on the injected material	Easy to perform High take rate Fast tumour development (2–4 weeks) Tumour growth can be easily monitored	TME not representative of orthotopic TME Immunodeficient mice needed for human material	221
Portal vein injection of cells or organoids	CRLIM	No primary tumour	–	Liver	High success rate Fast tumour development (2–4 weeks)	Challenging to perform No primary tumour	222,223
Splenic injection of cells or organoids	CRLIM	Spleen (if not resected)	Yes, if not resected; dependent on cell or organoid line	Liver; dependent on cell or organoid line	Relatively easy to perform (without resection) High success rate Fast tumour development (2–4 weeks)	Primary tumour is in the spleen (if not resected) Primary tumour growth or resection of the spleen might impact the immune microenvironment of the resulting CRLIM	223

Table 1 (continued) | Mouse models to study CRC progression

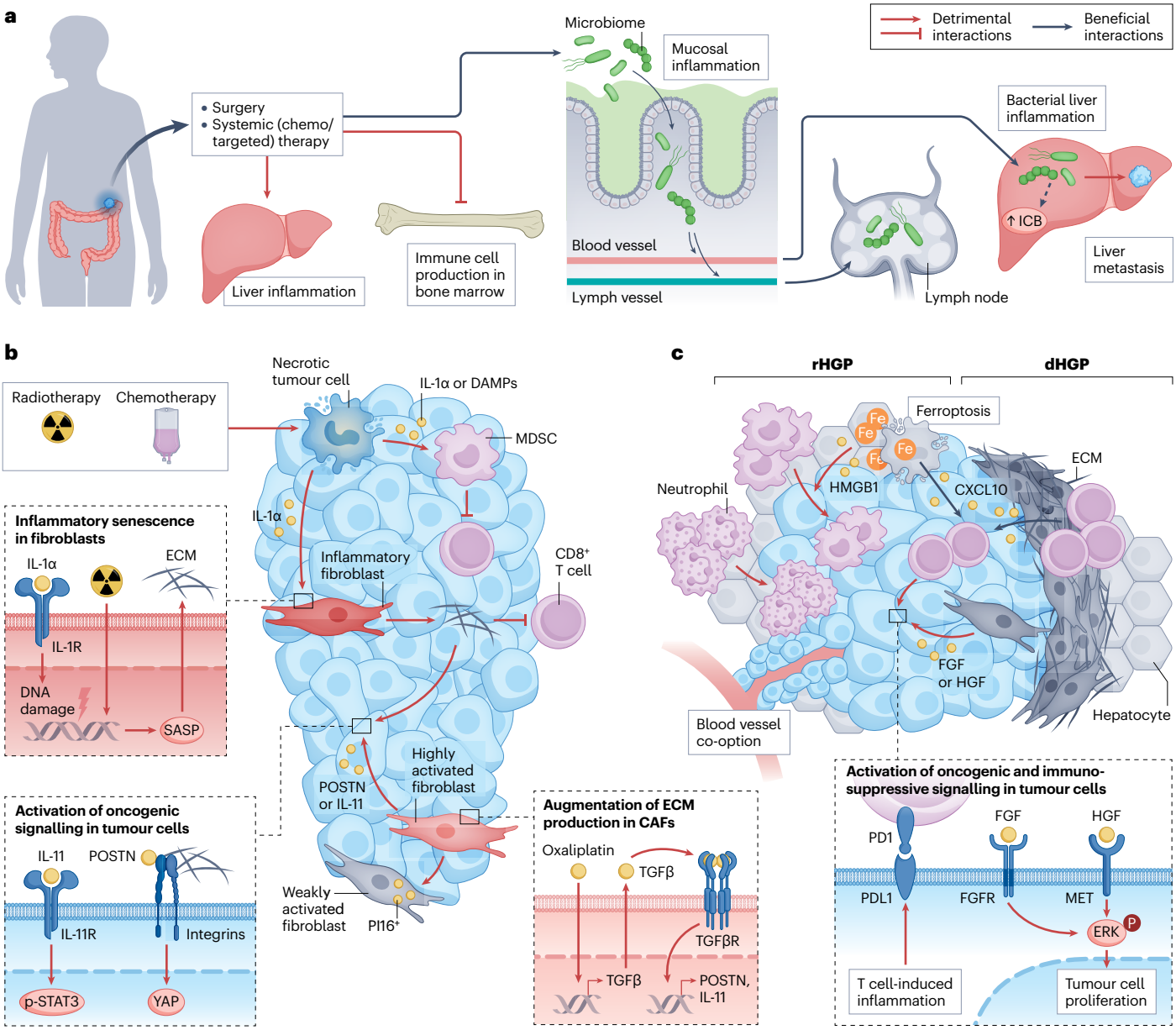
Mouse model	Type of CRC studied	Primary tumour location	Invasion of primary tumour	Metastasis	Advantages	Disadvantages	Example refs.
Injection of exogenous material (continued)							
Submucosal injection into the rectum or distal colon of cells or organoids	Sporadic CRC, CRLIM, colorectal lung metastasis	Colon Rectum	Yes	Liver Lungs Dependent on cell or organoid line	Fast tumour development (4–8 weeks)	Challenging to perform Does not capture first steps of invasion into the mucosa	222
Submucosal injection into the caecal wall	Sporadic CRC, CRLIM, colorectal lung metastasis	Caecum	Yes	Liver Lungs Dependent on cell or organoid line	Relatively easy to perform Fast tumour development (2–4 weeks) Resection of the primary tumour is possible	Requires laparotomy Does not capture first steps of invasion into the mucosa The microbiome is not representative of the remaining colon	203
Tail vein injection	Colorectal lung metastases	No primary tumour	–	Lung	Easy to perform	No primary tumour	223
Intraperitoneal injection	Peritoneal metastases	No primary tumour	–	Peritoneum	Easy to perform	No primary tumour	224

This table is adapted from ref. 225, Springer Nature Limited, and represents a non-exhaustive list of mouse models that were chosen based on their usage in important studies in the field. AOM/DSS, azoxymethane/dextran sulfate sodium; APC, APC regulator of WNT signalling pathway; CRC, colorectal cancer; CRLIM, colorectal liver metastasis; MSS, microsatellite stable; PDX, patient-derived xenograft; TGFβ, transforming growth factor-β; TME, tumour microenvironment.

an impaired intestinal barrier function and translocation of bacteria to mesenteric lymph nodes and the liver[144]. Intriguingly, in a mouse model of CRC, dissemination of bacteria from the colon to the liver leads to augmented expression of TGFβ and an increased presence of myeloid cells, creating a pre-metastatic hepatic niche which thus promotes CRLIM growth[145]. Additionally, chemotherapy in patients with CRC has also been shown to alter the composition of the gut microbiome[146], which may be relevant for secondary treatment with ICBs as the presence or absence of several microbiota strains has been associated with impaired responses to ICB in both human and mouse CRC[10]. Taken together, the effects of chemotherapy on the systemic immune microenvironment require further investigation as they may modulate subsequent antitumour immunity and immunotherapies[143].

In locally advanced rectal cancer (LARC) (a definition that includes advanced stage II and all stage III rectal cancers), preoperative (or neoadjuvant) treatment using radiotherapy and chemotherapy is the standard of care and aims at downsizing the tumour to enable complete surgical resection, reduce the risk of local recurrence and eradicate distant micrometastases[147]. Intriguingly, in a mouse model of LARC, an IL-1 receptor 1 (IL-1R1)-dependent inflammatory fibroblast subpopulation undergoes cellular senescence upon neoadjuvant radiotherapy which promotes therapy resistance and exclusion of cytotoxic T cells[148]. This effect could be reverted by the administration of the recombinant IL-1R1 antagonist anakinra[148], and the potentially synergistic effect of anakinra and chemoradiotherapy in patients with LARC is currently being investigated in a clinical trial[149,150]. Conversely, a complete response to neoadjuvant chemoradiotherapy in patients with LARC is associated with the expansion of a PI16$^+$ steady-state fibroblast subset and CD8$^+$ T effector memory cells, which could suggest that successful cytotoxic therapies lead to normalization of the CAF phenotype, which in turn allows an infiltration of T cells to attack the remaining tumour cells[151]. Similarly, in a large single-cell study of patients with dMMR or MSI-H CRC treated with neoadjuvant ICB, a complete response was associated with a resolution of myeloid inflammation, a reduction of IL-1β$^+$ monocytes and a concomitant increase in CD8$^+$ T effector memory cells[152]. This influx of CD8$^+$ T cells may be due to the indirect effect of ICB on endothelial cells, as combination immunotherapy in mouse models of CRC was shown to induce differentiation of tumour endothelial cells into high-endothelial venules fostering an influx of T cells into the tumour[153]. Mechanistically, the transformation into high-endothelial venules was promoted through activation of NF-κB in endothelial cells by lymphotoxin-α and lymphotoxin-β stemming from CD8$^+$ T cells that entered the tumour during the early phase of successful ICB[153].

Lastly, in mCRC (stage IV), treatment with various combinations of chemotherapy and targeted therapies is carried out to either establish surgical resectability of the metastatic lesions or to prolong patient survival. In CRLIM, treatment-induced microenvironmental changes have been suggested to be important for patient prognosis. For example, in a large single-cell study of human CRLIM, lesions that did not respond to therapy had a substantially increased proportion of neutrophils compared with responsive lesions[123]. Such an effect of systemic therapies and response patterns on the immune composition in CRLIM has also been noted by other studies[154–156] and needs to be considered when interpreting data from human CRLIM samples, given that most patients receive preoperative systemic treatment with chemotherapy and targeted therapy. Intriguingly, preoperative treatment can give rise to a desmoplastic histological growth pattern (dHGP) of resected CRLIM that is characterized by an increased stromal cell content at the metastasis–liver interface, increased T cell densities and a positive prognosis[157,158]. The coexistence of extensive fibrosis and anti-tumorigenic adaptive immune cells in CRLIM of the dHGP suggests that, in contrast to primary CRC, fibrosis might be beneficial in CRLIM. Conversely, the lack of a desmoplastic reaction at the metastasis–liver interface of CRLIM displaying the prognostically detrimental replacement histological growth pattern (rHGP) could imply that depletion or suppression of some TME components is a sign of highly aggressive tumours. This might also explain why the efficacy of several TME-targeted therapies including anti-VEGF,



anti-VEGF receptor (anti-VEGFR) and combinatorial receptor tyrosine kinase inhibitors with ICB depends on the presence of a stromal reaction in patients with mCRC pMMR or MSS[159,160]. Moreover, the accumulation of oncogenic mutations renders mouse and human CRC cells insensitive to stromal cues[161], as exemplified by KRAS mutations that confer resistance to epidermal growth factor receptor (EGFR)-targeted therapies in patients[162]. Thus, one could hypothesize that targeting central oncogenic pathways in tumour cells is a prerequisite for successful TME-directed therapies of immune-cold, late-stage CRCs.

Clinical implications of the CRC immune microenvironment

Results from recent clinical studies have corroborated the enormous potential of ICB for the treatment of patients with dMMR and MSI-H CRC. A comprehensive overview of recruiting and completed studies of different neoadjuvant ICB regimens in CRC is presented elsewhere[163]. For example, nivolumab (anti-PD1) together with ipilimumab (anti-CTLA4) improves progression-free survival of patients with dMMR or MSI-H mCRC, with 72% of patients in the ICB group compared with only 14% in the chemotherapy-treated control group showing stable disease at 24 months[6]. In patients with dMMR or MSI-H LARC, neoadjuvant treatment with the dostarlimab (anti-PD1) resulted in a complete response of the primary tumour in almost all patients[164]. More recently, neoadjuvant treatment of dMMR CRC with nivolumab plus ipilimumab or with nivolumab plus relatlimab (anti-LAG3) reported a complete response in approximately 70% of patients[165,166]. However, few patients with pMMR or MSS CRCs respond to immunotherapies and identifying those patients who are likely to benefit from ICB is challenging[167,168]. Given the role of the

Fig. 5 | Mechanisms of therapy-induced modulation of the CRC immune microenvironment. a, Surgery and chemotherapy lead to systemic and hepatic inflammation[247] that is, in part, driven by translocation of gut microbiota to the liver[144,247] and lymph nodes following a transient reduction of the intestinal barrier. Bacterial hepatic inflammation promotes growth of colorectal liver metastasis (CRLIM), yet could also improve the efficacy of immune checkpoint blockade (ICB) (dashed line). Additionally, most chemotherapeutics impair the production of immune cells in the bone marrow, which could impair the efficacy of immunotherapies. **b**, Neoadjuvant treatment of primary colorectal cancers (CRCs) results in tumour cell death and release of damage-associated molecular patterns (DAMPs) and interleukin-1α (IL-1α) that attract immunosuppressive myeloid-derived suppressor cells (MDSCs) and polarize cancer-associated fibroblasts (CAFs) towards an inflammatory phenotype. Irradiated inflammatory CAFs undergo senescence and secrete extracellular matrix (ECM) molecules that promote tumour growth[248] and T cell exclusion[82], thus conferring therapy resistance. In contrast, response to neoadjuvant treatments can result in the normalization of CAFs from a fibroblast activation protein (FAP^+) myofibroblastic to a $PI16^+$ steady-state phenotype. The chemotherapeutic oxaliplatin reinforces transforming growth factor-β (TGFβ) signalling in CAFs to increase production of periostin (POSTN) and IL-11, both of which have been shown to foster CRC growth via signal transducer and activator of transcription 3 (STAT3) and yes1-associated protein (YAP) signalling[51,249]. **c**, In CRLIM, ferroptotic cell death of hepatocytes attracts MDSCs as well as $CD8^+$ T cells, leading to upregulation of PDL1 on tumour cells which can be overcome by simultaneous MDSC blockade and ICB[211]. A desmoplastic histological growth pattern (dHGP) is associated with successful chemotherapy and/or targeted therapy, increased prognosis and an influx of beneficial immune cells compared with a replacement histological growth pattern (rHGP)[157]. Likewise, in patients who initially responded to systemic therapy with cetuximab, an expansion of CAFs was described which provides fibroblast growth factors (FGFs) and hepatocyte growth factor (HGF) to sustain tumour cell proliferation, thereby promoting secondary therapy resistance[155]. Similarly, chemotherapy in non-responders can lead to an influx of pro-tumorigenic neutrophils, as a rHGP of CRLIM associates with vessel co-option that confers resistance to some therapies, including anti-vascular endothelial growth factor (anti-VEGF) therapies[159]. CXCL10, C–X–C motif chemokine ligand 10; FGFR, fibroblast growth factor receptor; HMGB1, high mobility group box 1; IL-1R, IL-1 receptor; SASP, senescence-associated secretory phenotype; TGFβR, TGFβ receptor.

tumour and systemic immune environment in dictating response to various types of treatment, profiling the immune reaction could provide predictive and prognostic biomarkers for stratifying both patients with dMMR or MSI-H CRCs and patients with pMMR or MSS CRC[168–171] (Table 2). For example, systematic quantifications of $CD3^+$ and $CD8^+$ T cells in the tumour core and invasive margin, a method termed the 'immunoscore', could allow for prognostic stratification of CRC and could guide therapeutic decision-making[172]. Patients with CRC with a high tumoural infiltration of $CD3^+$ and $CD8^+$ T cells (that is, a high immunoscore) have a better prognosis, regardless of the clinical disease stage[72,156]. Moreover, in retrospective analyses of two cohorts of patients with LARC, the immunoscore of pretreatment tumour biopsies predicted disease recurrence in those patients who underwent neoadjuvant systemic therapy and showed a complete response[169], suggesting that the immunoscore might help select those patients who are eligible for a watch-and-wait strategy. Indeed, in a randomized clinical trial, scoring of $CD3^+$ and $CD8^+$ T cell densities in the tumour core and invasive margin of resected stage II and stage III CRCs helped define low-risk and high-risk patients with regard to 2-year recurrence rates[170]. Although relative reductions in disease recurrence rates as a consequence of adjuvant chemotherapy were similar between high-risk and low-risk groups, the a priori low disease recurrence rates in patients with a beneficial tumour immune contexture compared with those in high-risk patients (6.6% versus 23.5% 2-year recurrence rates in the selected study) might aid in the decision of whether or not a patient should receive adjuvant chemotherapy. As such, this highlights how immunity-based biomarkers might reduce overtreatment in CRC[170]. Moreover, given that a notable proportion of tumours with high T cell infiltration or immunoscore are pMMR or MSS CRCs[72], it is not implausible that those patients with pMMR or MSS tumours that are highly infiltrated by T cells could also respond to immunotherapy. Indeed, several studies that have investigated the efficacy of ICB in pMMR or MSS CRCs show that markers of adaptive immunity in tumour tissues including $PD1^+CD8^+$ T cells and the spatial distribution of $CD8^+$ T cells and $PDL1^+$ tumour cells are predictive of a response to ICB[168,171]. This suggests that $CD8^+$ T cell-related biomarkers could help select those patients who respond to ICB despite having pMMR or MSS tumours. In fact, there is accumulating evidence that combining ICB with conventional radiochemotherapy for the preoperative treatment of patients with pMMR or MSS LARC substantially increases the fraction of complete responders from 10–30% to 40–50%[147,173,174]. More specifically, in one study a transcriptomic signature of cytotoxic lymphocytes along with an increased fraction of $PDL1^+$tumour cells in pretreatment biopsies was predictive of a response[173].

Using more granular approaches such as the 'immune subtype classification', which considers the reactivity of $CD8^+$ T cells towards the tumour cells based on single-cell expression data, can help distinguish tumour-suppressive from bystander $CD8^+$ T cell activity on a transcriptomic level. This allows the identification of immune landscapes that have a positive prognostic value and associate with response to ICB[175]. Moreover, the inclusion of bacterial signatures can help refine immune microenvironment-based biomarkers. For example, the mICRoScore is a composite score based on T helper 1 cell-related and cytotoxic T cell-related gene expression and a microbiome signature driven by *Ruminococcus bromii* that can identify patients with CRC and good prognoses[17]. Lastly, the presence of tertiary lymphoid structures (TLSs), in both colorectal primary tumours and CRLIM, correlates with an improved prognosis[176,177]. Moreover, in CRC, TLSs associate with the presence of $CXCR5^+CD8^+$ exhausted progenitor T cells which are important for successful treatment with ICB in several cancers[178,179]. Therefore, the presence of TLS might serve as a positive prognostic and predictive biomarker for ICB treatment in patients with CRC.

Assessing tissue-based biomarkers necessitates invasive procedures such as biopsies or surgery and does not capture the entire complexity of a tumour or inform about systemic alterations of the immune system[180]. Hence, blood-borne and radiological biomarkers and a combination thereof might be better to guide therapeutic decisions. In patients with CRC, a higher MHC class II-related gene signature of circulating $CD8^+$ T cells prior to treatment with ICB correlated with an improved patient outcome[181]. Conversely, a high blood neutrophil-to-lymphocyte ratio, an indicator of chronic systemic inflammation and an adverse prognostic factor in various cancer entities[182], is associated with an impaired response to several standard CRC targeted therapies including bevacizumab (VEGF monoclonal antibody)[183],

Table 2 | Immune biomarkers in CRC

Biomarker	Clinical stage	Outcome	Refs.
$CD3^+$ and $CD8^+$ T cells at the invasive front and in the tumour core	I, II, III, IV	Better overall survival (when detected in the primary tumour or CRLIM)	72,156
$CD8^+$ T cell/T_{reg} cell ratio[a]	IV	Better overall survival	226
$CD8^+PD1^+$ T cells	I, II, III, IV	Better response to ICB in patients with pMMR or MSS	168
CMS4	I, II, III, IV	Worse overall survival (when detected in primary tumour), unaltered overall survival (when detected in CRLIM)	9,227
Decorin	I, II, III, IV	Worse progression-free survival, worse response to ICB	148,228
dHGP	IV (CRLIM)	Better overall survival	157
dMMR or MSI-H	I, II, III, IV	Better response to ICB	166,167
Fat-laden macrophages (foam cells)	I, II, III, IV	Worse disease-free survival	229
$IL4I1^+$ macrophages	I, II, III	Better overall survival	101
NKG2D ligands	I, II, III	Better disease-specific survival	230
Neutrophil-to-lymphocyte ratio[a]	I, II, III, IV	Worse overall survival, disease-specific survival and progression-free survival Worse response to bevacizumab, regorafenib and ICB	182–186
rHGP	IV (CRLIM)	Worse overall survival	157
$SERPINB2^+$ macrophages	IV	Better disease-free survival	128
$SPP1^+$ macrophages	I, II, III, IV	Worse overall survival	101,103,104
$THBS1^+$ monocyte-like cells	I, II, III, IV	Worse disease-specific survival	231
TLS	I, II, III, IV	Better overall survival	176,232
Short distance between $CD8^+$ T cells and $PDL1^+$ tumour cells	IV	Better response to ICB in patients with CRC with pMMR or MSS	171

CMS4, consensus molecular subtype 4; CRC, colorectal cancer; CRLIM, colorectal liver metastasis; dHGP, desmoplastic histological growth pattern; dMMR, mismatch repair-deficient; ICB, immune checkpoint blockade; MSI-H, microsatellite instability-high; MSS, microsatellite stable; pMMR, mismatch repair-proficient; rHGP, replacement histological growth pattern; SERPINB2, serpin family B member 2; SPP1, secreted phosphoprotein 1; THBS1, thrombospondin 1; TLS, tertiary lymphoid structure; T_{reg} cell, regulatory T cell.
[a]These biomarkers are assessed in the blood of patients whereas all other biomarkers are tumour tissue-based.

regorafenib (a multi-receptor tyrosine kinase inhibitor)[184] and ICB in patients with MSS and MSI-H CRCs and associates with an adverse prognosis[182,185,186]. Although the exact mechanisms for this are not clear, some immunosuppressive features of neutrophils and an increase of certain circulating MDSC subtypes (which are captured and counted as neutrophils during routine clinical laboratory testing) could play a role[187,188]. Accordingly, the occurrence of a certain subtype of MDSCs in the blood of patients with mCRC before treatment is linked to reduced progression-free survival after chemotherapy[189,190]. Lastly, the presence of immunosuppressive adaptive immune cells such as $CD4^+$ T_{reg} cells and a reduced cytotoxic $CD8^+$ T cell to T_{reg} cell ratio in the peripheral blood of patients with mCRC prior to therapy is associated with worse progression-free and overall survial[191,192]. This together highlights the potential of circulating immune cells as prognostic and predictive biomarkers in CRC.

Given the high impact of the stromal compartment and, specifically, CAFs for CRC pathogenesis and prognosis[9,148], it is not surprising that CAF markers also associate with therapy resistance in patients with CRC. For example, a high expression of the CAF-derived proteoglycan decorin in pretreatment biopsies correlated with decreased progression-free survival in a cohort of patients with LARC who were treated with neoadjuvant therapy[148]. Similarly, the CAF-rich CMS4 in primary tumours of patients with LARC and patients with mCRC associates with resistance to radiotherapies and chemotherapies[193,194]. Notably, CAFs in CRC were recently shown to accumulate oxaliplatin upon treatment, which reinforces TGFβ signalling in CAFs and ultimately fosters therapy resistance. Moreover, a signature of oxaliplatin-retaining CAFs correlated with an inferior outcome in patients with CRC[195]. This study serves as one explanation for the clinical observation that oxaliplatin-based regimens show a reduced effectiveness in the treatment of CMS4 mCRC compared with other chemotherapeutic regimens[194]. In another study, a single-cell RNA sequencing-derived gene expression signature of $IL\text{-}1R^+$ CAFs was associated with reduced overall survival of patients with CRC[196]. Conversely, high gene expression and serum levels of the endogenous IL-1R antagonist (IL-1RA) before preoperative chemoradiotherapy correlated with a better prognosis in patients with CRC, suggesting that $IL\text{-}1R^+$ CAFs could be mediators of therapy resistance in CRC[148]. Lastly, circulating fibrosis markers such as collagens and their cleavage products associate with worse overall survival in patients with mCRC who have been treated with chemotherapy and bevacizumab[197]. Given that the same fibrosis markers also associate with response to ICB in patients with melanoma, it is likely that they could also be used to predict the efficacy of ICB in CRC[198].

Conclusions and future directions

Although the success of ICB in the treatment of dMMR and MSI-H CRC[6] demonstrates that some subtypes of CRC can be targeted by immunotherapy, the lack of success in treating patients with pMMR and MSS CRC[167] underscores the need for new approaches. To make these patients eligible for immunotherapy, strategies must be developed that overcome immunosuppression in advanced pMMR and MSS CRC while enhancing their immunogenicity. As the latter, in part, relies on the quantity and presentation of tumour antigens, vaccination against individual or common CRC antigens could be a means to augment the antitumour immune response. Recently, the patient-specific tumour antigen mRNA vaccine autogene cevumeran, together with atezolizumab (anti-PDL1), delayed relapse in patients with pancreatic cancer after surgical resection of the primary tumour. This vaccine is currently being investigated as an adjuvant treatment in patients with stage II and stage III CRC[199,200]. Similarly, postoperative administration of a peptide vaccine targeting both G12D-mutated and G12R-mutated KRAS in resected pancreatic cancers and CRCs resulted in promising recurrence-free survival in the three out of five patients with CRC

Glossary

Acute-phase response
(APR). The APR of the liver is triggered by pro-inflammatory cytokines in response to infection, tissue injury, trauma or other stressors and is characterized by, among other alterations, changes in plasma protein concentrations.

Azoxymethane/dextran sulfate sodium (AOM/DSS) model
A mouse model that gives rise to inflammation-associated tumours in the colon through injection of AOM, a potent mutagen, followed by repeated cycles of mucosal inflammation induced by DSS.

Enteric glia cells
Cells that ensheath neurons of the enteric nervous systems to protect them from damage and support their functions.

Hepatic stellate cells
Fibroblast-like cells in the liver that populate the perisinusoidal space (located between hepatocytes and liver sinusoidal endothelial cells) and secrete collagens and other extracellular matrix (ECM) molecules upon activation.

Hepatocytes
The parenchymal cells of the liver that perform diverse metabolic functions including plasma protein synthesis, carbohydrate metabolism and storage, detoxification and bile acid secretion.

Liver sinusoidal endothelial cells
Cells that form the inner lining of the liver sinusoids and regulate the exchange of nutrients, metabolites and toxicants between the blood and hepatocytes.

Mitophagy
A process by which old or damaged mitochondria are degraded intracellularly.

Relapsing–remitting inflammation
A type of chronic inflammation with acute flares and phases of only mild inflammatory activity in between.

Steatosis
Abnormal build-up of fat in cells and organs, most commonly in the liver, where it is also known as fatty liver disease.

Tertiary lymphoid structures
(TLSs). Clusters of B lymphocytes and T lymphocytes that develop within inflamed or cancerous tissues and exhibit several hallmark characteristics of secondary lymphoid organs such as germinal centres, T cell-rich zones and high endothelial venules.

Tumour buds
Small clusters of cells at the invasive front of colorectal cancers (CRCs) that are not connected to the remainder of the tumour.

who had a strong vaccine response[201]. A consecutive trial testing the efficacy of a related vaccine targeting seven common KRAS and NRAS mutations is underway[202]. However, whether cancer vaccines will be effective in the setting of clinically overt CRC metastases where immunosuppression and exclusion of T cells are more prevalent compared with micrometastases[203] remains an open question. Additionally, vaccination approaches rely on the tumour cell's capacity to present tumour antigens via MHC class I and downregulation or loss of MHC class I expression is a common means by which CRCs evade immunity[204]. Hence, strategies aimed at MHC-independent tumour cell killing, such as chimeric antigen receptor (CAR)-T cell therapy or antibody-based approaches, might be more suitable for a broad group of patients. For example, CAR-T cells targeting molecules on the surface of tumour cells could be promising tools for the treatment of advanced CRC[205], yet several obstacles including the immunosuppressive TME, which inhibits CAR-T cell migration into and persistence within tumours, must be overcome[206]. Furthermore, new antibody-based drugs such as bispecific T cell engagers (BiTEs) that target molecules on the surface of tumour cells and activate T cells in direct proximity to facilitate MHC-independent tumour cell killing are auspicious candidates for further investigation. Cibisatamab, a BiTE targeting CEA on tumour cells and CD3 on T cells, together with atezolizumab showed some clinical activity in a cohort of heavily pretreated patients with MSS mCRC[207]. Likewise, the multifunctional Fc-enhanced anti-CTLA4 antibody botensilimab, together with the anti-PD1 antibody balstilimab, produced durable responses in patients with MSS mCRC[208]. Intriguingly, patients with active CRLIM showed no responses, again emphasizing the role of immunosuppression in CRLIM[208]. Therefore, strategies that target the immunosuppressive niche in the liver, for example lentivirus-driven hepatic upregulation of the pro-inflammatory cytokine IFNα[209] or blockade of immunosuppressive cytokine IL-10 (ref. 210), should be prioritized. Furthermore, induction of ferroptosis in hepatocytes together with ICB and MDSC blockade retarded growth of CRLIM but not subcutaneous tumours in a mouse model of MSS CRC, suggesting that changes specifically found in the hepatic TME could be hijacked for treating CRLIM[211].

Together, technological advances in drug development and novel insights into the context-dependent mechanisms and markers of immunosuppression from spatial investigations of human tumour tissue will be key for the development of successful immunotherapies for CRC. In this context, approaches involving artificial intelligence (AI) will likely be helpful to select patients with CRC who are eligible for immunotherapy. For example, a recent study demonstrated that the MSI status could be predicted from routine histology slides using deep learning with clinical-grade performance[212]. AI will also greatly facilitate the integrated analyses of heterogeneous types of data including clinical parameters, omics data and results from radiologic or blood testing, thereby supporting the development of new (immuno)therapeutic algorithms for CRC. Apart from these clinical considerations, additional preclinical work on the mechanisms of immunotherapy resistance must be carried out, and suitable in vitro and animal models must be developed for studying pMMR and MSS CRC, as most of the currently used cell lines and mouse strains do not adequately reflect the intricacies of human pMMR and MSS CRC. Innovative models including patient-derived tumour organoids, humanized mice, tissue slices, and organ and tumour-on-a-chip technologies will serve as valuable and complementary tools in the future for identifying novel therapeutic strategies[213].

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