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Reshaping the colorectal cancer immune microenvironment: insights from single-cell and spatial omics

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Abstract

Colorectal cancer (CRC) remains a significant global health challenge, ranking among the leading causes of cancer-related morbidity and mortality. Accumulating evidence indicates that disease progression and therapeutic resistance in CRC are not solely tumor cells but are critically shaped by the tumor microenvironment (TME), including immune and stromal compartments and the extracellular matrix. Recent advances in single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics (ST) have enabled the integration of cellular identity with spatial context, thereby defining spatial niches and interaction networks that modulate both antitumor immunity and tumor-promoting programs in CRC. This review synthesizes scRNA-seq and spatial transcriptomic studies of CRC to characterize cellular heterogeneity and to delineate spatial niche organization and functional crosstalk within the TME. Building on these insights, we summarize emerging therapeutic perspectives in CRC, including rational combination immunotherapy strategies and promising targets such as SPP1^{+} tumor-associated macrophages.

Keywords Colorectal cancer, Single-cell RNA sequencing, Spatial transcriptomics, Tumor microenvironment

Background

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy and the second leading cause of cancer-related deaths worldwide [1–3]. Recent advances in immunotherapy have demonstrated clinical benefit in CRC patients [4–8]. Immune checkpoint blockade (ICB), particularly targeting the programmed death-1 (PD-1) pathway, has shown promising clinical benefit in specific patient subsets. However, only approximately 20% of patients respond favorably to such therapies, primarily those with mismatch repair-deficient (dMMR) or microsatellite instability-high (MSI-H) tumors [9, 10], whereas most patients with microsatellite-stable (MSS) disease derive limited benefit [6, 11].

This therapeutic disparity is largely attributed to the tumor microenvironment (TME), a highly heterogeneous

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and immunosuppressive niche, encompassing immune cells, stromal elements, vasculature, extracellular matrix, and soluble factors [12, 13]. In CRC, the immunosuppressive TME can limit immunotherapy efficacy through interconnected mechanisms, including the recruitment and expansion of regulatory immune cell populations, the formation of physical and metabolic barriers that exclude effector T cells, and the secretion of inhibitory cytokines that dampen anti-tumor responses. Therefore, elucidating the cellular composition, spatial architecture, and functional states of TME components is critical for dissecting mechanisms of treatment resistance and identifying novel therapeutic targets in CRC.

Traditional bulk transcriptomic and flow cytometric analyses have provided valuable insights into the TME. However, these methods average signals across cell populations, thereby obscuring the cellular diversity and functional complexity of individual cell types [14, 15]. The advent of single-cell RNA sequencing (scRNA-seq), which allows for the analysis of gene expression at the resolution of single cells, has revolutionized our ability to deconstruct this complexity by enabling high-resolution analysis of individual cells within the TME [16–20]. More recently, spatial transcriptomics (ST), which measures gene expression while preserving tissue spatial context, has emerged as a complementary technology. Through mapping of cell-cell interactions and spatial gradients of signaling activity within intact tissue sections, spatial transcriptomics reveals how the spatial organization of the TME influences tumor progression and immune evasion [21].

This review synthesizes recent findings from scRNA-seq and spatial transcriptomics studies in human CRC, with an emphasis on the characterization of novel immune and stromal cell subsets, their spatial interactions and the key signaling pathways driving tumor progression and therapeutic resistance. These insights are reshaping our understanding of CRC pathobiology. More importantly, they provide a foundation for developing innovative therapeutic strategies aimed at reprogramming the TME to improve patient outcomes.

Search strategy and study selection

We searched the Gene Expression Omnibus (GEO) database for human scRNA-seq and spatial transcriptomic datasets relevant to CRC through 1 December 2025, using CRC-related terms ("colorectal cancer", "CRC") combined with technology-related terms ("single-cell RNA sequencing", "scRNA-seq", "spatial transcriptomics"). No restrictions were applied to publication year or dataset release date, and searches were limited to Homo sapiens. Studies were included if they profiled human CRC tumors or matched normal tissue using scRNA-seq or spatial transcriptomics. Mouse datasets were considered separately when offering complementary mechanistic insights. Studies focusing on non-CRC malignancies, or lacking single-cell or spatial data, were excluded.

Macrophages

Macrophages are sentinel immune cells that maintain tissue homeostasis, defend against pathogens, and orchestrate both innate and adaptive immune responses. In physiological conditions, tissue-resident macrophages carry out key functions such as phagocytosis (e.g., *C1QA*, *C1QB* and *C1QC*), antigen presentation, and immune regulation (e.g., *IL1B*, *NR4A1*, *NR4A2* and *NR3A3*) [22]. In the TME, macrophages represent one of the most abundant immune populations and are collectively referred to as tumor-associated macrophages (TAMs). TAMs have been implicated in tumor progression by regulating tumor cell proliferation, angiogenesis, immune suppression, and metastatic dissemination [23, 24].

Recent scRNA-seq studies have greatly refined our understanding of the macrophage landscape in human CRC [25]. Rather than conforming to the classical M1 (pro-inflammatory) or M2 (anti-inflammatory) paradigm, TAMs in CRC frequently co-express gene signatures associated with both M1 and M2 phenotypes, reflecting functional and transcriptional heterogeneity driven by local microenvironmental cues. Based on gene expression profiles, functional states, and spatial localization, TAMs can now be classified into distinct subpopulations that contribute differentially to immune regulation and tumor dynamics. The key characteristics of TAM subpopulations in CRC are summarized in Table 1. However, it is important to note that marker-based classifications do not always predict function uniformly across disease stages, MSS tumors, or anatomical sites, and emerging evidence suggests context-dependent plasticity within these subsets.

Functional subtypes of TAMs in CRC: immunosuppressive vs. inflammatory vs. anti-tumor

SPP1$^+$ TAMs are among the most frequently observed subsets, identified in hypoxic and necrotic regions of human primary CRC by scRNA-seq analysis. They are characterized by the expression of angiogenic and immunomodulatory genes (e.g., *VEGFA*, *VCAN*, and *CXCL8*) [26]. Their abundance increases progressively from normal to primary tumor to colorectal liver metastases (CRLM) regions [27]. It is noteworthy that most SPP1$^+$ TAMs originate from circulating classical monocytes with pro-inflammatory properties, while a subset derives from tissue-resident macrophages in CRC [28]. Furthermore, ICB therapy has been shown to reduce both the proportion of monocyte-derived SPP1$^+$ TAMs and their

Table 1 TAM subpopulations in CRC

Subset	Key markers	Platform	Species	Site	Spatial niche	Functions	Clinical association
SPP1⁺ TAMs	*SPP1*, *VEGFA*, *VCAN*, *CXCL8*	scRNA-seq	Human	Primary CRC, CRLM	Hypoxic or necrotic regions	Angiogenesis, immune suppression	Poor survival
TREM2⁺ TAMs	*TREM2*, *MARCO*, *APOE*, *MSR1*	scRNA-seq	Human	Primary CRC	Intratumoral	Immune suppression	Poor survival
C1QC⁺ TAMs	*C1QC*, *C1QA*, *CD68*, *CD80*	scRNA-seq, ST	Human	Primary CRC	Invasive front, peritumoral	T cells recruitment and activation	Better prognosis and ICB response
NLRP3⁺ TAMs	*NLRP3*, *IL18*, *ICAM1*, *IDO1*	scRNA-seq	Human	Primary CRC	Diffuse or neutrophil-clustered	Pro-inflammatory	Context-dependent effects
MRC1⁺CCL18⁺ TAMs	*MRC1*, *CCL18*, *TNF*, *IL1B*	scRNA-seq	Human	Primary CRC, CRLM	metastatic niches	Pro-inflammatory	Poor survival
IL4I1⁺ TAMs	*IL4I1*	ST	Human	Primary CRC	Fibrotic and apoptotic margins (uppermost mucosa)	Phagocytosis of tumor cells	Favorable prognosis
FOLR2⁺ TAMs	*FOLR2*	ST	Human	Normal colon, CRC	Middle or lower mucosal layers	Contribute to the plasma cell niche formation	Favorable prognosis
LYVE1⁺ TAMs	*LYVE1*, *IL10*	ST	Human	Normal colon, CRC	Submucosa, perivascular	Anti-inflammatory, fibrosis suppression	Unknown

output of inflammatory cytokines, suggesting that they may contribute to immune suppression [27].

SPP1⁺ TAMs exert context-dependent immune-suppressive effects through extensive interactions with other cell types in the TME. Specifically, SPP1⁺ TAMs activate FOXP3⁺ regulatory T cells (Tregs) in close proximity at primary and metastatic sites. In addition, SPP1⁺ TAMs can secrete high levels of IL-10, sustaining their activated state via autocrine feedback. Additionally, transcriptomic profiling reveals that SPP1⁺ TAMs exhibit elevated expression of genes linked to tumor growth, metastasis, and therapeutic resistance (e.g., *SDC2*, *S100A6*, *S100A8*, *S100A10*, *MT2A*, *MT1E*, and *MT1F*). Notably, in human CRLM, SPP1⁺ TAMs show markedly increased ligand-receptor interactions with tumor cells, including CD44-HBEGF, CD74-MIF, CD47-SIRPA, and TNFRSF1B-GRN. The crosstalk between SPP1⁺ TAMs and tumor cells may promote metastatic growth and immune evasion [29]. TCGA and GEO cohorts showed a positive correlation between SPP1⁺ TAMs and poorer survival outcomes, as well as resistance to immunotherapies in CRC [30, 31].

Another immunosuppressive subset, TREM2⁺ TAMs, are characterized by high expression of *TREM2* and lipid metabolism abnormalities [23]. The TREM2⁺ macrophages are specifically enriched within tumor tissues and highly express genes related to M2 macrophage activation (e.g., *MARCO* and *TREM2*), immune suppression (e.g., *HAVCR2*), lipid metabolism (e.g., *MSR1*, *APOC1*, *APOE*, and *OLR1*), and foam cell formation, all of which associated with tumor progression [32]. Interestingly, TREM2⁺ TAMs range from small mononuclear cells to large foam cells or multinuclear giant cells morphologically, reflecting its high degree of heterogeneity.

Chronic inflammation is a hallmark of TME. Inflammatory macrophage subsets have also been identified in human primary CRC by scRNA-seq. Specifically, NLRP3⁺ TAMs were identified with high expression of inflammatory cytokines (e.g., IL-18, ICAM1, IDO1, and iNOS) and the activation of inflammasomes in primary tumors. These cells are typically found either diffusely distributed throughout the tumor or clustered with neutrophils, suggesting they may help establish inflammatory niches by different mechanisms [33]. Notably, NLRP3 inflammasome activation exerts dual effects in CRC. IL-18 release can enhance natural killer (NK) cell-mediated cytotoxicity and has been linked to protective effects in colitis-associated CRC, whereas sustained IL-1β production promotes tumor-associated inflammation, facilitates epithelial-mesenchymal transition, and is associated with poor prognosis in sporadic CRC [34, 35]. The clinical relevance of NLRP3⁺ TAMs in human CRC and whether their inflammasome activity represents an anti-tumor or pro-tumor signal likely depend on disease stage, duration of activation, and the balance between IL-18 and IL-1β signaling.

Another notable inflammatory population is the MRC1⁺CCL18⁺ TAM subset, present in both primary CRC and CRLM. These macrophages upregulate pro-inflammatory mediators (e.g., *TNF*, *IL1B*, *CCL3*, and *CCL4*), amino acid metabolism genes (resembling Kupffer cells), and M2 macrophage polarization-related genes (e.g., *APOE* and *MARCO*) [30]. Importantly, this subset interacts preferentially with metastatic tumor cells via the CD47-SIRPA axis, which inhibits their phagocytic capacity. Remarkably, both SPP1⁺ and MRC1⁺CCL18⁺ TAM subsets decline significantly following neoadjuvant chemotherapy (NAC), a shift accompanied by a

concurrent increase in effector CD8⁺ T cells, may helping restore effective anti-tumor immunity [30]. Moreover, whether the decline in these TAM subsets is a cause or consequence of chemotherapy-induced tumor remodeling remains unclear.

On the other hand, C1QC⁺ TAMs represent a specific macrophage subset in human CRC, characterized by expression of M1 macrophage polarization-related markers (e.g., *CD68*, *CD80*, and *MAF*) and complement-related genes (e.g., *C1QA*, *TREM2*, *MERTK*, and *CD80*). However, their primary function appears to be the regulation of anti-tumor T cell responses. This population facilitates recruitment and activation of diverse T cell subsets, including CD8⁺ effector T cells, Tregs, and Th1-like cells, via extensive receptor-ligand interactions. Through this immunostimulatory activity, C1QC⁺ TAMs may contribute to enhanced anti-tumor immunity activity and have been associated with improved patient outcomes in human CRC [5]. However, emerging and conflicting evidence indicates the role of C1QC⁺ TAMs is context dependent. C1q-related programs have also been linked to tumor cell adhesion, migration, angiogenesis, and disease progression in certain settings, which correlate with unfavorable prognosis in multiple cancers, including CRC [36, 37]. Together, available evidence supports functional heterogeneity among C1QC⁺ TAMs and suggests context-dependent roles shaped by tumor subtype, spatial niche, and microenvironmental cues, highlighting the need for validation in larger, MSI-stratified, clinically annotated CRC cohorts.

Spatial organization of TAMs

Beyond transcriptomic heterogeneity, the spatial localization of TAMs is closely linked to their functional specialization [38]. Under physiological conditions, TAMs exhibit a zonated distribution across mucosal layers [33]. For example, IL4I1⁺ TAMs are found in the uppermost mucosal layer and retain their phagocytic capacity even in the tumor microenvironment. These cells preferentially localize to fibrotic or apoptotic tumor margins, where they phagocytose tumor cells, correlating with favorable prognosis. Their activity may be modulated by the CD274-CD47 axis. In the middle and lower layers, FOLR2⁺ TAMs contribute to the formation of the plasma cell niche. Additionally, the LYVE1⁺ TAM subpopulation located in the submucosa of the colon, surrounding blood vessels, resembles murine perivascular macrophages. The subpopulation specifically expresses *IL-10* and *LYVE1* and may contribute to suppress inflammation and fibrosis [38, 39].

Within tumors, TAMs display pronounced spatial heterogeneity. Macrophages at the invasive front often display anti-tumor features, whereas those located deeper in the tumor core adopt immunosuppressive or pro-metastatic functions [24]. A ST study using 10x Genomics Visium revealed that immunomodulatory TAMs (Reg-TAMs) localize primarily to stromal and peritumoral regions, regulating immune cell infiltration [21]. Conversely, tumor-core TAMs engage in direct cell-cell interactions that may promote invasion and metastasis [24]. Further supporting this differential localization, transcriptome profiling revealed that CD68⁺ macrophages infiltrate and tightly surround tumor cells, while CD163⁺ macrophages are preferentially located further from the tumor nests [33].

In colorectal liver metastasis, two morphological subclasses have been described: large TAMs (L-TAMs), which display a foam cell-like morphology with high cholesterol metabolism and phagocytic gene expression (e.g., *C1QA*, *C1QB*, *C1QC*, *MERTK*, *MSR1*, and *MRC2*), and small TAMs (S-TAMs), which express inflammatory markers. Notably, L-TAMs are particularly associated with poor prognosis, highlighting the potential clinical significance of TAM diversity in metastatic CRC [40].

Spatial transcriptomics has also facilitated mechanistic insights into TAM function. For example, SPP1⁺ TAMs, initially identified by scRNA-seq, have been found to co-localize with FAP⁺ fibroblasts by spatial transcriptomics, which contribute to immune exclusion by constructing fibrotic barriers that physically impede T cell infiltration [41]. These interactions are regulated by chemerin, TGF-β, and IL-1β, which may underlie resistance to PD-L1 blockade in patients expressing high levels of *FAP* and *SPP1*(23).

A recent study employed spatially resolved multi-omics approaches, including imaging mass spectrometry, spatial proteomics, and spatial transcriptomics, to elucidate the spatial TME of human metastatic CRC patients receiving immunotherapy. The study revealed that, regardless of microsatellite status, C1QC⁺ TAMs are more abundant in responders, where they co-localize with CD4⁺ T cells and the expression of MHC-II facilitates their interaction. In contrast, in non-responders, cancer-associated fibroblasts can inhibit this interaction [42].

These spatial distinctions highlight the importance of context-dependent TAM phenotypes and underscore the complexity of macrophage-mediated regulation within the CRC microenvironment.

Therapeutic targeting of TAMs in CRC

Given their central role in shaping tumor immunity, TAMs represent attractive therapeutic targets in CRC. However, clinical efforts have encountered several challenges. For instance, CSF1R inhibitors, while effective at depleting proliferative, inflammatory macrophages, have shown limited clinical efficacy in CRC. This may be due to their inability to eliminate immunosuppressive subsets

such as SPP1⁺ TAMs [28]. More promising strategies have focused on intracellular signaling pathways. SHP2, a phosphatase that negatively regulates type I interferon (IFN-I) signaling, has emerged as a viable target. SHP2 inhibition enhances anti-tumor immune responses and synergizes with immune checkpoint blockade in preclinical CRC models [43]. Additionally, dual blockade of TREM2 and PD-1 has demonstrated efficacy in murine CRC models. This approach depletes M2-like TAMs, promotes neutrophil expansion, and reprograms the remaining TAM populations toward a pro-inflammatory phenotype. These findings collectively highlight the need for more precise, subset-specific macrophage-targeting strategies in CRC, which are ideally guided by scRNA-seq and spatial transcriptomic profiling, to overcome immunotherapy resistance and improve clinical outcomes (Fig. 1).

Neutrophils

Neutrophils, traditionally conceptualized as short-lived first responders within the innate immune system, mediate rapid host defense via phagocytosis, degranulation, and the generation of reactive oxygen species (ROS). The N1/N2 framework proposed by Fridlender [44] was subsequently extended to tumors, depicting tumor-associated neutrophils (TANs) as either antitumor (N1, IFN-β-induced) or protumor (N2, TGF-β-driven) [45–47]. However, this simplistic binary dichotomy has been fundamentally challenged by the advent of scRNA-seq. Rather than conforming to strictly polarized states, TANs in CRC span a broad transcriptional continuum. Within this landscape, multiple functionally divergent subsets emerge, exhibiting unexpected capacities such as antigen presentation and metabolic regulation [48–50], positioning them as context-dependent orchestrators of the TME. These insights recast neutrophils not as mere terminally differentiated innate effectors, but rather as dynamic lineages that integrate and respond to complex microenvironmental cues.

This functional heterogeneity is exemplified by the four distinct TAN subsets identified by Bai et al., which arise from a progressive differentiation cascade orchestrated by spatially defined microenvironmental cues [48]. In normal colonic mucosa, NANs preferentially express *MS4A6A*, *AREG*, and *GPR183* and are regulated by TFs such as RFX5, CREB1, SRF, STAT4, and REL. These cells facilitate immune regulation, differentiation, and controlled inflammation to maintain tissue homeostasis. As tumorigenesis progresses, a stepwise differentiation path is initiated, wherein NANs transfer to

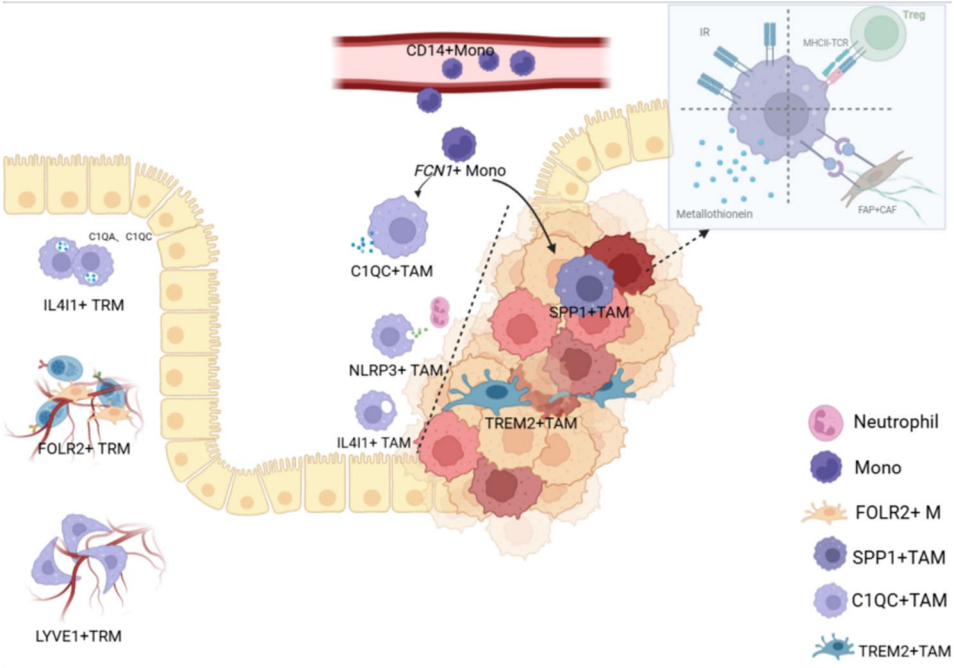

Fig. 1 Dissection of major tumor-associated macrophage subpopulations in the tumor microenvironment using multi-omics approaches. IL4I1⁺ TAMs, primarily involved in phagocytosis, retain their function in the tumor microenvironment. FOLR2⁺ TAMs contribute to plasma cell niche formation, while LYVE1⁺ TAMs, localized around blood vessels, specifically express *IL-10* and *LYVE1* to regulate inflammation and tissue fibrosis. C1QC⁺ TAMs are associated with complement activation and enhance antigen presentation to T cells. SPP1⁺ TAMs, located in the ischemic and necrotic regions of tumors, express inhibitory receptors, induce physical barrier formation, activate suppressive cells and limit the infiltration and function of cytotoxic cells. NLRP3⁺ TAMs are macrophages contribute to the inflammatory milieu within tumors. TREM2⁺ TAMs mediate immunosuppression, lipid metabolism through scavenging modified lipoproteins and foam cell formation, while also promoting Treg activation and complement activation

TAN-1. This subset exhibits a highly pro-inflammatory, matrix-degrading phenotype characterized by the secretion of matrix metalloproteinases (MMPs), potentially facilitating tumor cell invasion. Subsequently, TAN-3 emerges, primarily involved in immune activation and regulation, presumably through direct interactions with T cells. Concurrently, TAN-4 integrates metabolic and inflammatory programs to maintain immune balance within the TME, collectively contributing to an antitumor response. Finally, TAN-2 represents a terminally differentiated state, exhibiting a pronounced stress-tolerant phenotype that enables survival under hypoxic and nutrient-deprived TME conditions. This subset correlates with inferior overall survival, reflecting its pivotal role in shaping an immunosuppressive TME. Consequently, the abundance and functional characteristics of TAN-2 serve as a reliable proxy for the degree of immunosuppression within the TME. Targeting TAN-2 and its intrinsic stress-response machinery may disrupt neutrophil-mediated adaptation, enhance treatment efficacy, and prolong patient survival. Consistent with this concept of context-dependent plasticity, Wang et al. using spatial transcriptomics revealed that C3-TANs preferentially localize at the normal-adenoma interface and exhibit strong crosstalk with fibroblasts. This subset exhibited a functional shift from degranulation-dependent cytotoxicity toward protumorigenic, pro-angiogenic, and immunomodulatory activities along the normal-adenoma-carcinoma sequence, mirroring the emergence of stress-tolerant, immunosuppressive TAN states [51].

In addition, a newly identified subpopulation of $CTCF^+$ TANs, enriched in hypoxic tumor regions, orchestrates tumor progression and metastasis via the hypoxia-induced CTCF-MIEN1-IL-1β axis. These pro-tumorigenic neutrophils are strongly associated with inferior clinical outcomes and primary resistance to ICB. Targeting the CTCF-MIEN1-IL-1β signaling in the mouse model of CRLM enhanced the efficacy of anti-PD-L1 therapy, suggesting this pathway as a promising therapeutic target [52]. Moreover, neutrophils also play special roles in metastatic CRC. Preclinical orthotopic murine models of CRLM show an early $Prok2^+$ neutrophil expansion during pre-metastatic niche formation. However, the clinical relevance of analogous $Prok2^+$ neutrophil dynamics in human CRC patients remains to be rigorously determined [53].

Beyond their cellular heterogeneity, neutrophils exacerbate CRC pathogenesis through the formation of neutrophil extracellular traps (NETs), composed of decondensed chromatin decorated with antimicrobial proteins (histones, elastase, myeloperoxidase). In CRC, NETs are enriched at tumor invasive fronts, perivascular niches, and metastatic foci, triggered by pro-inflammatory cytokines (IL-8, TNF-α) and ROS. Consistently, TAN- related transcriptional signatures correlate with disease aggressiveness and patient outcomes [49, 54].

Collective insights from scRNA-seq and spatial transcriptomic technologies have highlighted neutrophil heterogeneity in CRC and their roles in TME remodeling, immune suppression and metastasis, but their spatiotemporal plasticity is still not fully defined. Mechanistic studies and neutrophil-based biomarkers could facilitate therapeutic targeting and improve patient stratification.

Dendritic cells

Dendritic cells (DCs) are pivotal antigen-presenting cells that play a critical role in inducing and maintaining antitumor immunity. Although they constitute a relatively small fraction of immune cells in the TME, they exert substantial immunomodulatory functions. Traditionally, DCs are categorized into classical dendritic cells (cDCs) and plasmacytoid dendritic cells (pDCs) [55–59]. cDCs can be further subdivided into $CD141^+$ cDC1s and $CD1c^+$ cDC2s. While cDC1s are primarily responsible for activating $CD8^+$ cytotoxic T cells, cDC2s are more involved in the activation of $CD4^+$ helper T cells. In contrast, pDCs are primarily involved in antiviral responses and the modulation of other immune cells, such as T cells and cDCs [60–62].

In CRC, the distribution of DC subsets is notably altered: pDCs are significantly increased and cDC1s markedly reduced [28]. While pDCs contribute to both inflammatory responses and anti-cancer immunity, their immunosuppressive effects are frequently more pronounced in human CRC [63]. In particular, pDCs have been shown to promote the expansion and differentiation of Tregs, a process associated with immunosuppression and unfavorable clinical outcomes [64] However, recent evidence from Kießler et al., based on immunohistochemical analyses of large human colon cancer cohorts, has challenged this monolithic view. They reported that elevated pDC infiltration, specifically when pDCs spatially co-localize with $CD8^+$ T cells within mature tertiary lymphoid structures (TLSs), correlates with significantly improved overall survival [65]. These divergent findings underscore that pDC function is strictly contingent upon their transcriptional activation state and precise spatial localization, highlighting the context-dependent complexity of their role in the CRC landscape. In contrast, cDC2s in CRC exhibit functional heterogeneity, giving rise to multiple subpopulations with distinct roles [31]. For instance, a distinct cDC2 subpopulation found in human CRLM is characterized by the expression of pro-inflammatory genes (e.g., *C1QA*, *CD68*, *CD163*, *CD14*, *VSIG4*, *CCL3,* and *MAFB*). This subpopulation closely resembles the recently described DC3 population in both human and murine models, and its enrichment is associated with poor prognosis in CRC. Additionally, $TIMP1^+$

cDC2 cells, identified in peritumoral liver metastases and mesenteric lymph nodes, express maturation markers like *CCR7* and participate in regulating angiogenesis (e.g., *EREG*, *CREM*, and *VEGFA*) [31].

A specialized subset of DCs, termed LAMP3$^+$ DCs, which are also referred to as mature regulatory DCs (mregDCs), has been identified in both human primary CRC lesions and CRLM [66]. LAMP3$^+$ DCs are defined by high expression of maturation markers (e.g., *CD40*, *CD80* and *CD86*), regulatory molecules (e.g., *PD-L1* and *BTLA*), migration-related genes (e.g., *CCR7*), and low expression of Toll-like receptor (TLR) signaling genes [25]. Lineage tracing and transcriptomic profiling have revealed that LAMP3$^+$ DCs originate from both cDC1 and cDC2 populations, with a predominance of cDC1-derived cells [25, 67]. However, the functional differences between cDC1- derived and cDC2-derived LAMP3$^+$ DCs remain unexplored, and direct functional studies are lacking.

LAMP3$^+$ DCs contribute to priming naïve CD8$^+$ T cells. High-resolution spatial profiling reveals their co-localization with CD8$^+$ T cells and CXCL13$^+$ helper T cells in TLS. Intriguingly, these TLS-resident DCs exhibit significantly increased *IL12B* and reduced *CD274* (*PD-L1*) expression compared to their counterparts localized within stromal regions. However, these cells also possess immunosuppressive properties, including the induction of Tregs via upregulation of *BTLA*, contributing to immune tolerance [68]. This dual-functionality mirrors the behavior of LAMP3$^+$ DCs observed in lung cancer, which similarly promote Treg differentiation [67].

Recent studies highlight that LAMP3$^+$ DCs are core components of a specialized immune triad, comprising CD8$^+$ T cells, CXCL13$^+$ helper T cells and LAMP3$^+$ DCs. This spatial organization, driven by IL-15 signaling, correlates with improved response to immune checkpoint blockade and may serve as a predictive marker for immunotherapy efficacy [69]. Mechanistically, LAMP3$^+$ DCs enhance anti-tumor immunity through IL-12 production and may modulate immune responses via *PD-L1* expression, depending on their activation state. Similar immunoregulatory dynamics have been observed in hepatocellular carcinoma, where LAMP3$^+$ DCs and CD4$^+$ T cell niches support effective CD8$^+$ T cell differentiation following PD-1 blockade [70]. A study using Stereo-seq revealed that the spatial architecture of LAMP3$^+$ DCs and their intercellular interactions with T cells distinguishing immunotherapy responders from non-responders. In responders, LAMP3$^+$ DCs form cohesive niches with CXCL13$^+$ T cells at the tumor-stroma invasive boundary, exhibiting higher spatial co-localization and interaction frequencies than in non-responders. Conversely, in non-responders, LAMP3$^+$ DCs are spatially segregated from T cells by an intervening barrier of CXCL14$^+$ CAFs [71]. Despite the robust association between these immunospatial niches and ICB outcomes [28], prospective validation and mechanistic studies are needed to establish causality and define CRC-specific functional plasticity of LAMP3$^+$ DCs.

T cells

T cells are central mediators of anti-tumor immunity and a focal point of immunotherapy research. Over the past decade, the advent of ICIs has deepened our understanding of the intricate regulation of T cell differentiation, governed by factors such as antigen exposure intensity and duration [72, 73]. Nevertheless, strategies to reverse dysfunctional or exhausted T cell states remain elusive. ScRNA-seq and ST now offer unprecedented resolution to map T cell heterogeneity and guide therapeutic innovation [73, 74].

Exhausted T Cell

T-cell exhaustion is a state of functional impairment that occurs in both chronic infections and tumors and represents a significant challenge to sustaining effective T-cell responses in cancer therapy [75, 76]. Exhausted T cells are typically characterized by co-expression of inhibitory receptors (e.g., *TIGIT*, PD-1, *LAG3,* and *CXCL13*) [77]. In CRC, exhausted T cells also exhibit high proliferative activity and express angiogenesis- and immunosuppression-associated genes (e.g., *CCL15*, *CXCL1*, and *CXCL12*) [73, 78]. Furthermore, these exhausted T cell populations accumulate to a greater extent in MSI-H patients, who tend to have a better response to immunotherapy.

Exhaustion is not a binary state but rather a continuum. Specifically, during the development of exhausted T cells, their transcriptional profile shifts from high expression of effector molecules (e.g., *IFNG*, *GZMB*, *GZMH*, and *PRF1*) to the accumulation of exhaustion markers and dysregulated lipid metabolism [73, 74, 78]. Given the continuous and heterogeneous nature of the T cell exhaustion process, it is challenging to comprehensively capture their dysfunctional state at a single time point. Recent scRNA-seq analyses introduced the concept of tumor-reactive CD8$^+$ T cells, which span from precursor exhausted T cells (Tpex) to terminally exhausted states [79]. It is particularly encouraging that effective ICB therapy can partially reverse the process of tumor-reactive CD8$^+$ T cells exhaustion and reactivate their anti-tumor function. In particular, the baseline infiltration level of this subpopulation can be a potential predictor of disease progression and outcome. Notably, recent studies have shown that reversing the reprogramming of lipid metabolism in failing T cells restores their cytotoxicity, and transcription factors such as *PPARG* and *SREBF2* are expected to be potential therapeutic targets [80].

In the intestinal mucosa, tissue-resident memory T cells (TRMs), another subset of exhausted T cells, have garnered particular interest. These cells show high expression of tissue-residency markers (e.g., *CD69* and *CD103*) and immune checkpoint molecule (e.g., *PD-1*) [81]. Unlike conventional exhausted T cells ($CD8^+$ Tex), TRMs still recover their immune function upon re-encountering the corresponding antigen, thus mediating a rapid immune response. The characteristics of TRMs in CRC have been associated with favorable clinical prognosis and highly effective therapeutic outcomes [22]. Interestingly, a recent study identified a subset of $CD8^+$ T cells expressing *GZMK*, but not *GZMB*. These $GZMK^+$ $CD8^+$ T cells exhibit minimal or no expression of typical exhaustion markers and have been shown to exert anti-tumor effects in CRC and other cancer types. This underscores the functional and origin-related heterogeneity among exhausted T cell populations and highlights the complexity of T cell-mediated immunity in cancer.

Regulatory T Cells

Tregs represent a key immunosuppressive population within the TME, complementing the role of exhausted T cells in shaping immune evasion. The accumulation of Tregs in the TME has been associated with poor prognosis and reduced sensitivity to treatment. Furthermore, anti-PD-1 therapy has been shown to reduce Treg cell numbers in responsive tumors [82]. The development and function of Tregs are primarily governed by the transcription factors *FOXP3* and *TCF-1*. In murine models of polyposis, deletion of *Tcf-1* impairs $CD4^+$ T cell polarization and promotes local inflammation, which exacerbates tumor progression in CRC [83]. In CRC patients, the majority of Tregs infiltrating the TME originate from peripheral circulation, driven by chemokine receptors such as *CCR4*, *CCR7,* and *CCR8* [84]. Thus, *CCR4* and *CCR8* represent targetable pathways for limiting Treg recruitment; however, CCR4 inhibition carries a risk of broader immune toxicity, whereas CCR8-directed strategies remain in early stage of development with limited clinical evidence in CRC [85]. However, a distinct subset arises from local $PRDM1^+$ TRM $CD4^+$ T cells, highlighting a dual origin [82]. Local inflammation within the tumor milieu provides critical cues for Treg activation.

Mechanistically, Tregs suppress anti-tumor immunity through multiple pathways. These include the secretion of inhibitory cytokines (e.g., IL-10, TGF-β), and contact-dependent engagement of immune checkpoint receptors, both of which contribute to the formation of an immunosuppressive barrier. Using 10x Genomics Visium spatial transcriptomics, Hashimoto demonstrated that tumor cells at the adenoma-carcinoma interface exploit the MDK-SDC4 axis to engage Tregs and promote immune suppression. Moreover, functional assays using MDK-knockdown tumor cells and SDC4-deficient Treg-like cells demonstrated reduced Treg chemotaxis, providing causal evidence that this axis actively drives Treg recruitment. In alignment with its functional role, an elevated ratio of MDK/SDC4 is clinically associated with poor overall survival in CRC patients. Thus, the MDK-SDC4 axis contributes to the formation of the Treg-enriched immunosuppressive microenvironment that fuels tumor progression [86]. Interestingly, another study identified a unique niche, termed the Treg-mregDC-lymphatic niche, in which $LAMP3^+$ DCs around lymphatic vessels recruit and activate Tregs via chemotactic signals. In $Villin^{Cre}$ $Apc^{fl/+}$ mice, multiplex immunofluorescence delineated a peritumoral lymphatic stromal compartment enriched for spatially co-localized Tregs and mregDCs. Functional intervention experiments further indicated a suppressive role for this axis, as CCR4 blockade or selective Treg depletion increased antigen trafficking to draining lymph nodes. Consistently, spatial transcriptomic analyses suggested analogous perilymphatic immune niches in human CRC, and their presence was associated with inferior patient survival, underscoring that immune-cell localization is a key determinant of function within the TME [87].

Tissue-specific heterogeneity of Tregs is also evident when comparing primary CRC tumors with liver metastases. In primary intestinal tumors, both $IL10^+$ and $CTLA4^+$ Treg subpopulations coexist and exhibit an activated and tissue-resident phenotype. By contrast, metastatic lesions in the liver predominantly harbor only the $CTLA4^+$ Treg subsets [31]. Notably, the $CTLA4^+$ Tregs from both sites share extensive TCR repertoire overlap with $FOXP3^+$ Tregs in the peripheral blood, indicating potential clonal relationships between tissue-infiltrating and circulating Tregs [86].

In addition to classical $FOXP3^+$ Tregs, CRC tissues also harbor a subset of $EOMES^+$ type 1 regulatory T (Tr1) -like cells. These unconventional regulatory T cells undergo clonal expansion in both primary and metastatic lesions and exhibit suppressive activity comparable to $FOXP3^+$ Tregs. They are characterized by the expression of *GZMK* and chitinase-3-like protein 2 (*CHI3L2*). Importantly, their accumulation has been associated with advanced disease progression and resistance to immunotherapy, underscoring their potential as novel therapeutic targets in CRC.

Conventional T cells

Compared to Tregs, conventional T cells (Tconvs) demonstrate significantly greater heterogeneity in both gene expression and T cell receptor (TCR) repertoires across various cancer types . In CRC, scRNA-seq has identified a distinct subpopulation of $CXCL13^+$ Th1-like T cells expressing *BHLHE40* and effector molecules such as

IFNG and *GZMB*. These cells are preferentially enriched in MSI-H patients and have been associated with improved responses to ICB, suggesting their potential utility as biomarkers for immunotherapy outcomes. Furthermore, CXCL13⁺ Th1-like T cells promote the activation of CD4⁺ memory T cells and facilitate robust *IFNG* production via *IGFLR1*, a costimulatory receptor recently identified to augment T-cell activation and effector cytokine production. These findings indicate that *IGFLR1* may serve as a promising therapeutic target to potentiate anti-tumor immune responses in CRC [84].

Overcoming T cell exhaustion and selectively eliminating immunosuppressive T cell subsets remain major obstacles in advancing effective anti-tumor immunity. Although scRNA-seq has provided unprecedented insights into the transcriptional states of tumor-infiltrating T cells, current spatial transcriptomic technologies have yet to fully resolve their spatial localization, functional states, and interactions within the TME [87]. Future studies should prioritize mapping spatially resolved signaling networks and cell-cell interactions that contribute to T cell dysfunction. Such efforts will be critical for the development of precise, TME-aware immunotherapies aimed at restoring the durability and cytotoxic functionality of tumor-reactive T cells within the immunosuppressive landscape of CRC [76].

B cells

In recent years, ICB and chimeric antigen receptor T-cell (CAR-T) immunotherapy have garnered significant clinical attention, underscoring the critical role of T cells in anti-tumor immunity. Despite their success, these therapies have also highlighted the inherent limitations and vulnerabilities of T cell-mediated responses against cancer. An increasing number of studies have shed light on the essential synergistic role of tumor-infiltrating B cells and plasma cells, collectively referred to as tumor-infiltrating B cells (TIBs) [88, 89].

TIBs have been found to have diverse functions (Fig. 2). As antigen-presenting cells (APCs), B cells interact with CD4⁺ T helper cells via the CD40-CD40LG axis, further activating cytotoxic T lymphocytes (CTLs) to eliminate tumor cells [27]. This interaction is notably enhanced in treatment-responsive groups, and similar findings have been observed in mouse models. In addition to antigen presentation, TIBs play a pivotal role in orchestrating the initiation and maintenance of adaptive immunity through chemokine pairs such as CCL19-CCR7, CXCL13-CXCR5, and CCL28-CCR10 [90]. Beyond those functions, TIBs can secrete cytokines that promote the formation and maturation of TLSs in tumors [26]. However, within tumor tissues, a simultaneous decrease in the number of TIBs, their proliferation index and MHC II expression suggests impaired antigen-presenting function and reduced immune cell recruitment, ultimately impairing their anti-tumor efficacy [26]. Moreover, another distinct subset, regulatory B cells (Bregs), is characterized by its ability to produce potent inhibitory cytokines, primarily IL-10 and TGF-β, which attenuate antitumor immunity by suppressing T-cell effector functions and promoting Treg expansion.

On the other hand, following antigenic stimulation, B cells differentiate into plasma cells (PCs), which clonally expand and secrete IgG or IgA antibodies. These antibodies exert their effects through antibody-dependent cellular cytotoxicity (ADCC), a mechanism in which immune effector cells, including NK cells and macrophages, recognize antibodies bound to target cells through Fc-γ receptors (FcγRs), which triggers the release of cytotoxic granules or facilitates phagocytosis. Spatial transcriptomics has underscored that B-cell-mediated humoral responses in CRC are governed by spatially driven functional compartmentalization. Specifically, TLS-associated IgG⁺ plasma cells and class-switched B cells are more often linked to anti-tumor immunity, whereas IgA⁺ PCs enriched in TLS-poor regions tend

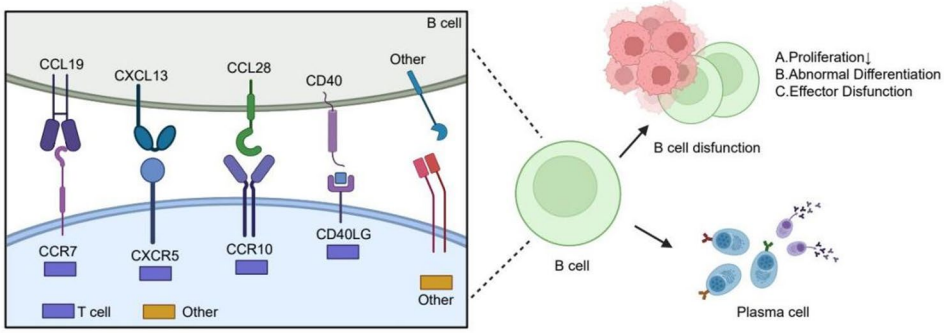

Fig. 2 The role of B cells in tumor microenvironment. B cells express various immune-regulatory molecules and participate in anti-tumor immunity. Upon antigen stimulation, B cells proliferate, differentiate and mature into PCs to exert anti-tumor functions. However, tumor-infiltrating B cells exhibit a reduced anti-tumor immune response, manifested as impaired proliferative capacity, along with compromised antigen presentation and recruitment abilities

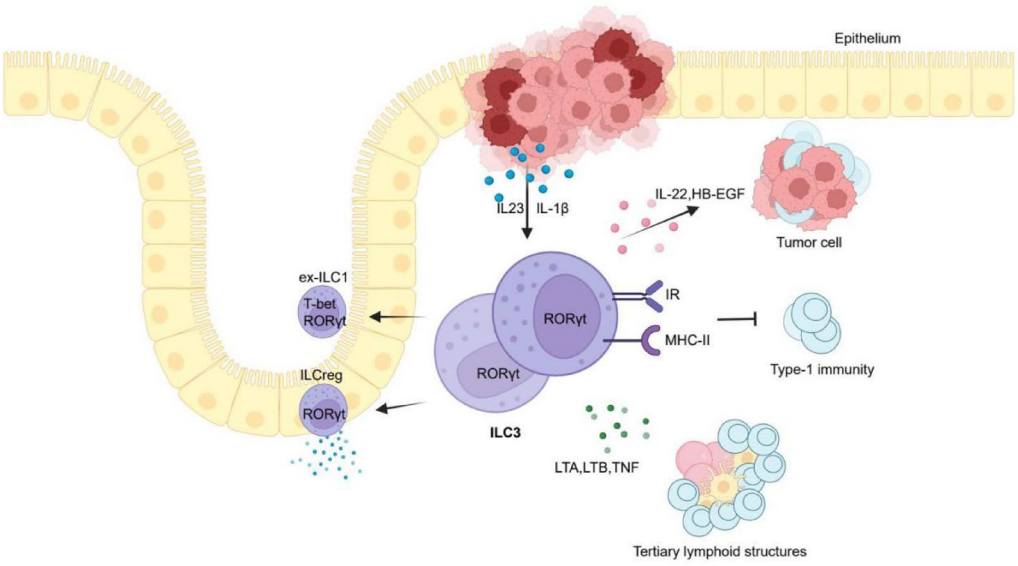

Fig. 3 ILCs participate in tumor progression through multiple mechanisms. Under the stimulation of inflammatory cytokines such as IL-23 and IL-1β, ILC3s secrete inflammatory cytokines that can exert either anti-tumor or pro-tumor effects, depending on the stage of tumor progression. ILC3s also express immune-regulatory molecules, regulating adaptive immunity. Furthermore, under the influence of environmental factors, ILC3s exhibit functional plasticity, undergoing remodeling to various extents in the TME

to support immunosuppressive and pro-tumor niches [90]. Additionally, a study reported that elevated levels of $IgA^{+}IGLC2^{+}$ plasma cells are associated with poor prognosis [91]. Notably, Erbin knockout reduces the secretion of TGF-β by IgA^{+} PCs, hindering tumor cell migration and inhibiting STAT6-mediated PD1 expression. These findings are consistent with previous research in lung cancer, where Erbin is shown to promote tumor progression [27].

ScRNA-seq has enabled the identification of specific TIB clonotypes and their associated phenotypes, shedding light on their antigen recognition and effector functions. However, B -cell-based immunotherapies encounter obstacles, such as the complexity of B-cell differentiation and the difficulty of selectively targeting tumor-associated B cells without affecting normal humoral immunity [92]. While the link between B-cell clonotype tracking and CRC prognosis is still underexplored, integrating multi-omics technologies could facilitate a more precise characterization of B-cell clonotypes, offering promising insights for the development of B-cell-targeted immunotherapies.

Innate lymphoid cells

Innate lymphoid cells (ILCs) is an important part of innate immunity, specifically located on the mucosal surface. While ILCs share a common lymphoid origin with B cells and T cells, they are inability to undergo RAG-mediated antigen receptor rearrangement, resulting in a lack of the specificity and diversity characteristic of TCR and BCR receptors [93, 94]. Based on the transcriptional profile and cytokines production, ILCs are classified into five groups: natural killer (NK) cells, ILC1s, ILC2s, ILC3s and lymphoid tissue inducer (LTi) cells. Their non-redundant roles in regulating intestinal physiology, immune tolerance, and mucosal homeostasis have been increasingly recognized [95, 96].

ILC3s are the major subsets in the intestinal mucosa and exhibit a wide range of homeostatic functions, including coordination of lymphoid tissue development, regulation of commensal bacteria, support of host defenses, modulation of adaptive immune responses and promotion of tissue repair. However, significant dysregulation of ILC3 occurs in response to a variety of microbial exposures and human diseases, including inflammatory bowel disease (IBD), food allergies, chronic infections and gastrointestinal cancers [97, 98]. During different stages of tumor progression and the various phases of adaptive immune defenses, ILC3s show diverse molecular expression patterns and participate in CRC development (Fig. 4). As innate analogs of Th17 cells, ILC3s can produce IL-22, which promotes intestinal epithelial regeneration, activates DNA damage response pathways and restrains inflammation during early tumorigenesis. While chronic inflammation persists, ILC3s can become a driver of cancer progression [94, 97]. In addition to participating in inflammatory regulation, ILC3s have been reported to regulate microbiota composition and support anti-tumor type 1 immunity through MHC molecules [99]. In the DSS-induced colitis mouse model, loss of PD-1 disrupts ILC3 homeostasis, dysregulates gut microbiota composition, reduces STAT3 activation, and

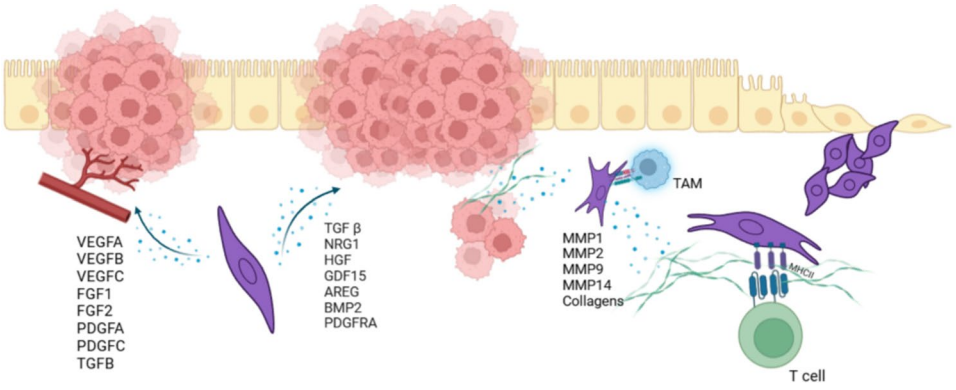

Fig. 4 Major functions of tumor-infiltrating fibroblasts in TME. ScRNA-seq revealed the diversity of CAF phenotypes, characterized by their involvement in various processes: supporting malignant epithelial growth, angiogenesis, immunosuppression, and participation in the epithelial-mesenchymal transition (EMT) process, contributing to tumor cell invasion

reshapes their metabolic programming. ILC3s have also been shown to express several co-stimulatory molecules (e.g., *OX40L*, *CTLA4*, and *ICOS*), though their relevance in immunotherapy remain unclear [97]. ILC3s are also crucial contributors to the formation and maturation of TLS within TME. These cells express TLS-inducing factors (e.g., *LTA*, *LTB*, and *TNF*) and *MHC-II* and selectively localize to the tumor microenvironment, particularly at the invasive edges, as observed in the AOM/DSS mouse model of inflammatory colorectal cancer. Interestingly, an inverse correlation was found between ILC3 abundance and TLS density, a complex spatially organized regulatory relationship that warrants further investigation [99, 100]. ILC3s exhibit significant plasticity in TME. In the $Apc^{Min/+}$ mouse model, TGF-β signaling has been shown to drive the transdifferentiation of intestinal ILC3s into regulatory ILCs (ILCregs), which acquire immunosuppressive functions by secreting cytokines such as interleukin-10 (IL-10). These ILCregs, marked by *IL10* and *Id3*, retain the expression of several ILC-associated surface markers (e.g., *Cd25*, *Il-2r*, *Sca-1*, and *Cd90*), but notably lack key transcriptional markers characteristic of Tregs (e.g., *Cd4* and *Foxp3*). Importantly, depletion of ILCregs has been shown to reverse their pro-tumorigenic effects, underscoring their potential as a therapeutic target. Additionally, tumor-infiltrating ILC3s exhibit a dynamic phenotypic shift during CRC progression. Specifically, these cells gradually increase the expression of ILC1-related markers (e.g., *CD39*, *CD244*, and *SIRPγ*), along with cytotoxicity-associated genes (e.g., *TIGIT*, *CD247*, *PRF1*, *GNLY*, *GZMA*, *GZMB*, *and GZMK*), while losing their characteristic ILC3 markers (e.g., *KIT* and *CD127*). This phenomenon is conserved in mice. Gregory F. et al. reported similar shifts in ILC phenotypes in the $APC^{min/+}$ mouse model. This transition suggests a functional reprogramming of ILC3s in the evolving tumor microenvironment, but the molecular mechanisms governing this plasticity remain largely unresolved and require further investigation [100].

Subpopulations of ILC1-like (marked by *KLRD1* and *TBX21*) and ILC2 cells (markered by *PTGDR2* and *BCL11B*) have also been identified in human CRC tissues, which are notably absent in normal colonic mucosa, suggesting a tumor-associated expansion or differentiation of these subsets. A study revealed that ILC1-like cells undergo a functional shift as the disease progresses, from an activated phenotype characterized by high expression of activating receptors (e.g., *Klrd1*, *Ncr1*, *Klrc2*, and *Klrb1c*), toward an immunosuppressive state marked by the upregulation of inhibitory receptors (e.g., *Klre1* and *Klra7*) in the AOM/DSS mouse model of inflammatory colorectal cancer. ILC1-like cells induce T cell dysfunction through the expression of the immune checkpoint *TIGIT*. Concurrently, ILC2-like cells upregulate multiple genes associated with immune suppression (e.g., *Hs3st1*, *Ctla2a*, *Ltb4r1*, *Pdcd1*, *Tnfrsf18*, and *Hes1*) and promote tumor growth through *PTGDR2* and IL-13, indicating a potential role in shaping a tumor-permissive microenvironment [100]. Notably, evidence from non-colorectal tumor types has revealed additional functional programs within the ILC2 that may provide conceptual insights for CRC. In lung cancer, a subset of ILC2s that specifically express granzyme B (*GZMB* has been identified, playing a distinct role in anti-tumor immunity [101, 102]. A recent study in pancreatic cancer also revealed that IL-33-activated ILC2s can induce the formation of TLSs [103].

NK cells play a critical role in immune surveillance, but their function is significantly suppressed by the TME in CRC. Zhang et al. identified two major heterogeneous NK cell populations: NK-KLRC1 cells and NK-GZMH cells. The former expresses *KLRC1*, *NKG2A*, *FCGR3A*, and *FGFBP2*, while secreting XCL1 and XCL2 to recruit $XCR1^{+}$ DCs. In contrast, NK-GZMH cells, enriched for *GZMH* and *PRF1*, represent a more cytotoxic-leaning

subset. Despite their distinct molecular profiles, both subsets exhibit profound immune dysfunction in tumor. This is characterized by the upregulation of inhibitory molecules such as *HAVCR2* and *LAG3* [22], aligning with functional studies that show impaired cytokine production and increased inhibitory-receptor expression in CRC-infiltrating NK cells [104]. In addition, a six-gene NK-associated signature, including *ADAM8*, *CTSD*, *CCL4*, *IL2RB*, *TTC38* and *PLEK*, has been proposed to predict prognosis and immunotherapy responsiveness in CRC [105].

Unlike their cytotoxic counterparts, ILCs primarily function as non-cytotoxic auxiliary sentinels that orchestrate the immune landscape rather than exerting direct tumoricidal effects. Consequently, the functional impact of ILCs is inherently tied to their spatial proximity to other immune and stromal constituents. Given the current lack of in situ data, integrating high-resolution spatial profiling is crucial to deciphering these intercellular communication hubs, ultimately guiding the design of ILC-targeted therapies that can effectively re-program the CRC environment.

Fibroblasts

Cancer -associated fibroblasts (CAFs), a major stromal component of the TME, play multifaceted roles in CRC progression. Multiple studies have reported that CAFs contribute to malignancy by promoting tumor proliferation, invasion, and therapeutic resistance through secretion of immunomodulatory factors, direct interaction with neighboring cells, and remodeling of the extracellular matrix (ECM) (Fig. 4) [69, 106, 107]. Advancements in scRNA-seq, have enabled the identification of canonical CAF markers, including *FAP*, *VCAN*, and *COL1A2* [108]. Recent studies have revealed critical functions of CAFs in promoting tumor cell proliferation through the secretion of a variety of factors, such as *TGF-β*, *NRG1*, *HGF*, *GDF15*, *AREG*, *PDGFRA*, *and BMP2* [66]. Beyond their structural roles, CAFs are pivotal orchestrators of T cell dysfunction in CRC. Specifically, a subset of MHC^+ CAFs characterized by the expression of antigen-presentation-related genes (e.g., *CD74* and *TAP1*), yet lacks the co-stimulatory molecules (e.g., *CD40*, *CD80*, and *CD86*) essential for full T cell activation has been identified. This incomplete antigen presentation may contribute to T cell anergy and immune evasion [26]. This immune evasion is further compounded by the subtype-specific deployment of checkpoint ligands, such as the *TIGIT-NECTIN2* axis between $CAFS1^+$ CAFs and $CD8^+$ T cells and the *LGALS9-HAVCR2* axis between ecm-myCAFs and $GZMB^+$ $CD8^+$ T cells [109]. However, emerging studies have identified functionally distinct CAF subsets with tumor-restraining or immune-permissive properties in CRC. For instance, NAC-enriched CAF_PI16 and CAF_SLIT2 subsets enhance anti-tumor immunity by secreting *CXCL12*, *SLIT2*, and *Decorin*, which collectively drive DC recruitment and bolster T-cell activity [110]. Additionally, a putative tumor-restraining CAF subset expressing *ADAMDEC1*, *CXCL14*, *EDNRB*, and *PROCR* has been associated with favorable prognosis and enhanced immunotherapy responsiveness [111].

Complementing these transcriptomic studies, spatial transcriptomics has provided crucial insights into the tissue localization and functional specialization of CAF subpopulations. For instance, $CTHRC1^+$ CAF subtype displays significant spatial colocalization with a specific malignant epithelial cell subset, with both residing within an EMT-active spatial niche. Mechanistically, $CTHRC1^+$ CAFs promote EMT and increase the invasiveness of adjacent malignant epithelial cells via a WNT5A-MSLN axis, as demonstrated in in vitro co-culture models [112]. $CXCL12^+$ fibroblasts, expressing mesenchymal markers (e.g., *DCN*, *SLIT2*, and *CXCL12*), are predominantly located in the lamina propria. Notably, CAFs can recruit $CD8^+$ effector memory T cells through the CXCL12-CXCR4 axis, which could be enhanced by ICB or NAC [110]. In addition, $CCL2^+$ fibroblasts, marked by *F3*, *WNT5A*, *BMP2*, *POSTN*, and *HSD17B2*, localize near the epithelial layer [27]. Additional spatial analyses have identified CAF subsets residing at the luminal margin (LM) and dilated blood vessels, promote angiogenesis and inflammation through the secretion of VEGFA and NRG1, particularly in MSI-H CRC tumors. Additionally, a distinct CAF population enriched at the LM edge, characterized by the expression of *MCAM*, was reported to modulate the generation of $CXCL13^+$ CD8 T cells through the JAG1-NOTCH1 axis [66]. These treatment responses-related CAF populations become activated and amplified under the influence of prostaglandin (PG) secreted by adjacent endothelial cells in the TME [110].

Given their immunosuppressive and tumor-supportive roles, CAFs have emerged as promising therapeutic targets in CRC. Current strategies focus on eliminating immunosuppressive CAFs or inhibiting CAF-derived factors in order to reprogram the CAF phenotype toward a more quiescent or tumor-restraining state. However, several challenges remain, including the absence of specific CAF biomarkers and the high degree of functional and spatial heterogeneity across CAF subsets [113]. Although several CAF subsets have been reported and systematically summarized (Table 2), further integration of scRNA-seq and spatial transcriptomics will be critical to resolve the functions of specific CAF population and guide the development of more precise and effective stromal-targeting therapies.

Table 2 Systematic characterization of CAFs

Subtype	Markers	Impact on TME	Therapeutic hypothesis or strategy	Ref.
ecm-myCAFs	*GJB; ANTXR1, SDC1*	Interact with GZMB$^+$ CD8$^+$ T cells via LGALS9-TIM-3 axis, inducing T cell exhaustion; interact with TAMs via TGFB1-TGFBR1/2 pairings, promoting cancer progression	Target LGALS9-TIM-3 axis	[109]
MHC-II$^+$ CAFs	*CD74, TAP1*	Exhibit incomplete antigen presentation due to absent co-stimulatory molecules (*CD40*, *CD80* and *CD86*), inducing T-cell anergy and tolerance.	Restore co-stimulatory signaling	[26]
CAFS1$^+$ CAFs	*CAFS1*	Interact with CD8$^+$ T cells via TIGIT-NECTIN2 axis, inducing T-cell exhaustion	Target TIGIT-NECTIN2 axis	[109]
CCL2$^+$ CAFs	*F3, WNT5A, BMP2, POSTN, HSD17B2*	Participate in pro-inflammatory and tumor-promotion processes, and decrease post-treatment.	NA	[27]
MCAM$^+$CAFs	*MCAM*	Modulate generation of CXCL13$^+$ T cells via JAG1-NOTCH1 axis.	Target JAG1-NOTCH1 axis	[66]
CAF_PI16	*PI16, DCN, CXCL12, SLIT2, C7, C3*	Enhance antigen processing and presentation in DCs, facilitating T-cell infiltration and activation	Preserve and enhance immune function afterNAC	[110]
CAF_SLIT2	*SLIT2, DCN, CXCL12, SLIT2, C7, C3*	Enhance antigen processing and presentation in DCs, facilitating T-cell infiltration and activation	Preserve and enhance immune-activating function	[110]
ADAMDEC1$^+$ CAFs	*ADAMDEC1, CXCL14, EDNRB, PROCR*	Are associated with favorable prognosis, and T-cell infiltration	Enhance function; serve as a biomarker for immunotherapy response	[111]
CTHRC1$^+$ CAFs	*CTHRC1*	Promoting EMT and enhance tumor invasiveness through the WNT5A-MSLN axis, driving CRC progression and metastasis	Target WNT5A-MSLN axis; inhibit EMT	[112]

Spatial immune axes in CRC: characterizing resistance-associated niches and future combinatorial immunotherapy

The TME of CRC is defined by a strict spatial organized, where the non-random positioning of immune and stromal cells dictates the trajectory of ICB response. While responders exhibit a transition toward permissive tissue architectures that facilitate T-cell penetration, non-responders are characterized by the maintenance of resistance-associated niches that keep CD8$^+$ T cells effectively locked at the invasive front [114]. This spatial sequestration is enforced by a composite barrier system: a physical scaffold of dense collagen and dysfunctional vessels, coupled with a biochemical milieu rich in inhibitory cytokines and checkpoint ligands secreted by CAFs and myeloid cells. Such a perspective necessitates a paradigm shift in our understanding of immunotherapy resistance: ICB failure is not merely the product of cellular exhaustion but is encoded within the spatial organization of the stroma. Consequently, future interventions must prioritize the topographical reprogramming of these niches to unleash anti-tumor immunity.

The most extensively characterized spatial resistance axis in CRC is formed by FAP$^+$ CAFs and SPP1$^+$ TAMs. These two populations tend to localize in the tumor regions and may communicate via TGF-β, IL-1β, and chemerin, where intratumoral CD8$^+$ T cell infiltration is reduced. Consequently, the spatial architecture of this FAP$^+$ CAFs-SPP1$^+$ TAMs serves as a robust histological signature of lymphocyte exclusion and is consistently associated with worse clinical outcomes across CRC cohorts [41, 42]. Beyond FAP$^+$ CAFs-SPP1$^+$ TAMs axis, spatial transcriptomics has identified additional CAF subtypes that construct distinct resistance niches through spatially organized mechanisms (3). CTHRC1$^+$ CAF displays significant spatial colocalization with a specific malignant epithelial cell subset, with both residing within an EMT-active spatial niche, where it functions as a primary driver of EMT and malignant invasion through WNT5A-MSLN axis [112]. Simultaneously, the COL10A1$^+$ CAF-M2 macrophage axis represents a critical immunosuppressive hub. COL10A1$^+$ CAFs orchestrate immunosuppression by activating JAK1/STAT3 signaling to induce M2 polarization. In turn, M2 macrophages upregulate the expression of *COL10A1* in CAFs via the TGF-β-RUNX2 pathway, forming the pro-tumorigenic COL10A1$^+$ CAFs-M2 macrophage axis [112]. Such spatially coordinated remodeling is even more pronounced in the metastatic regions. CRLM is defined by a unique recruitment of MRC1$^+$CCL18$^+$ TAMs, which establish an intercellular communication axis with tumor cells via CD47-SIRPA signaling, thereby fostering an environment conducive to metastatic outgrowth [29].

In stark contrast to these resistance-promoting hubs, the presence of C1QC$^+$ TAMs had emerged as a hallmark of ICB responsiveness, regardless of microsatellite status. However, the anti-tumor potential of these macrophages is not cell-intrinsic but strictly contingent upon the formation of structured spatial niches with CD4$^+$ T cells [42]. Multimodal profiling reveals that while close spatial proximity between C1QC$^+$ TAMs and CD4$^+$ T cells is a strong predictor of therapeutic success, this productive

Table 3 The summary of spatial immune axes and cell-cell interactions in CRC

Spatial axis/Cell pair	Key ligand-receptor/Signaling	Spatial localization	Functional effects	Clinical correlation	Refs
SPP1$^+$ TAMs and tumor cells	CD44-HBEGF, CD74-MIF, CD47-SIRPA, TNFRSF1B-GRN	Hypoxic and necrotic regions; markedly enriched in CRLM	Promote metastatic growth and immune evasion	Poor survival; resistance to immunotherapy	[29]
SPP1$^+$ TAMs and FAP$^+$CAFs	Chemerin, TGF-β, IL-1	Co-localize in tumor regions; construct fibrotic barriers	Immune exclusion; physically impede T cell infiltration	Poor survival; resistance to immunotherapy	[41]
MRC1$^+$CCL18$^+$ TAMs and tumor cells	CD47-SIRPA	Primary CRC and CRLM	Pro-inflammatory	Decline after NAC with a concurrent increase in CD8$^+$ T cells	[30]
C1QC$^+$ TAMs and CD4$^+$ T cells	MHC-II-mediated interaction	Co-localize in spatial niches (responders); separated by CAFs in non-responders	T cell recruitment and activation	Better ICB response	[42]
Tumor cells and Tregs	MDK-SDC4	Adenoma-carcinoma interface	Treg recruitment and chemotaxis	Poor survival	[86]
B cells and CD4$^+$ Th cells	CD40-CD40LG	Tumor	Activate cytotoxic T lymphocytes	Favorable prognosis	[27]
MHC$^+$ CAFs and T cells	MHC-II, CD74, TAP1 (lacking CD40, CD80, CD86)	Stromal regions	Incomplete antigen presentation leads to T cell anergy	Poor survival	[26]
CAFS1$^+$ CAFs and CD8$^+$ T cells	TIGIT-NECTIN2	Not specified	Induce T cell exhaustion	-	[109]
ecm-myCAF and GZMB$^+$ CD8$^+$ T Cells	LGALS9-HAVCR2	-	Induce T cell exhaustion	-	[109]
CXCL12$^+$ CAFs and CD8$^+$ Effector Memory T Cells	CXCL12-CXCR4	-	Recruit CD8$^+$ T cells	-	[110]
MCAM$^+$ CAFs and CXCL13$^+$ CD8 T cells	JAG1-NOTCH1	Luminal margin edge	Modulate generation of CXCL13$^+$ CD8 T cells	-	[66]
CTHRC1$^+$ CAFs and tumor cells	WNT5A-MSLN	EMT-active spatial niche	Promote EMT; increase invasiveness	Poor survival	[112]
COL10A1$^+$ CAFs and M2 Macrophages	JAK1/STAT3; TGF-β-RUNX2	Tumor regions	Immunosuppression; pro-tumorigenic axis	Poor survival	[112]

interaction is frequently thwarted in non-responders. In these cases, CAFs physically interpose between the two immune subsets, acting as a structural wedge that dismantles the responsive niche and enforces a state of therapeutic resistance.

Collectively, these observations indicate that immunotherapy response in CRC is encoded not simply by cell-intrinsic features but by spatially organized immune-stromal axes. Models that interrogate CAFs, myeloid cells or lymphocytes in isolation fail to explain why tumors with similar transcriptional programs show divergent ICB responses. Spatial transcriptomics provides the potential mechanistic explanation: resistance is sustained by redundant, spatially organized multicellular programs rather than any single suppressive cell type [115]. Framing the TME in terms of spatial axes elevates therapeutic resistance from a sum of isolated pathways to an emergent property of organized ecosystems. Spatial transcriptomics thus reframes the therapeutic question from "which cell to deplete" to "which niche-sustaining circuits to disrupt".

Preclinical evidence from murine CRC models underscores the transformative potential of deconstructing spatially defined resistance niches. In MC38/NIH3T3-FAP tumor-bearing mice, combining ^{177}Lu-labeled FAP-targeted radioligands with anti-PD-L1 antibodies achieves complete tumor eradication and durable immunity, with treated mice rejecting tumor rechallenge. Mechanistic analyses reveal that this combination therapy disrupted the FAP$^+$ CAF-mediated physical barrier and reprogrammed the TME by increasing CD8$^+$ T cell infiltration, enhancing mature antitumor neutrophils, reducing regulatory T cells [116]. These preclinical findings provide proof-of-concept that dismantling spatially organized stromal-immune barriers can restore ICB sensitivity in otherwise refractory MSS colorectal cancer.

However, translating these spatial insights into clinical benefit remains a key unmet challenge. Early-phase trials targeting stromal or myeloid compartments in CRC have shown limited efficacy, likely in part because patients are enrolled without spatially informed stratification. A central inference from spatial transcriptomics is that response depends on aligning an intervention with

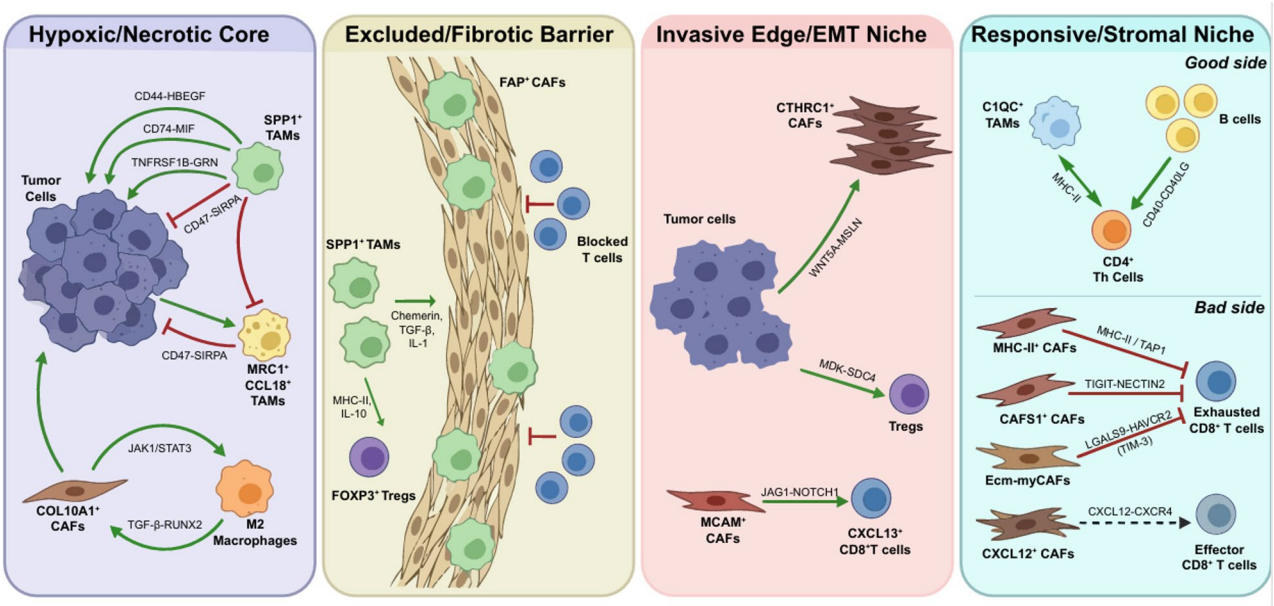

Fig. 5 Spatial immune axes and cell-cell interactions in CRC. Schematic overview of spatially organized immune niches within the colorectal cancer tumor microenvironment, illustrating how non-random positioning of stromal, myeloid, and lymphoid cells governs immune exclusion, dysfunction, or responsiveness. Hypoxic and fibrotic regions are dominated by immunosuppressive CAFs and TAMs that establish physical and biochemical barriers to T-cell infiltration, while invasive-edge niches engage EMT-associated stromal programs and spatially restricted immune interactions. In contrast, responsive immune-permissive regions support productive antitumor immunity, whereas adjacent CAF-enriched niches drive CD8⁺ T-cell exhaustion through inhibitory checkpoint and chemokine signaling

the dominant spatial architecture of an individual tumor; accordingly, future trials should prospectively enrich for tumors harboring the target niche using baseline spatial profiling and incorporate on-treatment readouts to verify niche disruption while preserving productive immune regions. Ultimately, the value of spatial transcriptomics is not simply to catalog TME heterogeneity, but to operationalize spatially stratified therapy as a precision framework in which treatment selection and sequencing are guided by the spatial programs that encode resistance. Realizing this goal will require prospective trials co-developed with spatial profiling platforms to move spatial omics from discovery to clinical decision-making in CRC (Fig. 5).

Conclusion

Despite substantial progress in emerging neoadjuvant therapies and immunotherapies [117], significant challenges persist in the treatment of CRC, primarily due to the inherent heterogeneity of tumors and the complexity of the TME. These factors continue to limit the effectiveness of current therapeutic strategies and underscore the need for more refined approaches.

Over the past decade, the advent of scRNA-seq and spatial transcriptomics has revolutionized our understanding of the TME [118–121]. By integrating the high-resolution transcriptomic profiling of scRNA-seq with the spatial contextualization offered by spatial transcriptomics, researchers have been able to precisely localize distinct cell populations and map intercellular interactions within the tumor niche. This dual approach has overcome limitations of earlier methodologies and provided unparalleled insights into the cellular composition, functional heterogeneity, and regulatory dynamics of tumor, immune, and stromal cells. Together, these technologies have significantly accelerated the discovery of novel biomarkers and informed the development of more effective, personalized cancer therapies.

Nonetheless, spatial transcriptomics remains an evolving discipline. Its broader adoption has been constrained by high costs and the limited resolution of many existing platforms, which often fall short in capturing the intricate cell–cell interactions within the TME at true single-cell resolution. Despite its transformative potential, the clinical adoption of spatial transcriptomics remains constrained by formidable economic barriers and the intrinsic resolution limits of early-generation platforms. Traditional capture-based technologies, exemplified by the 10x Genomics Visium assay (55 μm spot size), often yield mixed signals from heterogeneous cell clusters, obscuring the fine-scale cell-cell interactions within the TME. To address this, high-density innovations like Visium HD have pushed the boundaries toward near single-cell granularity (2 × 2 μm), though their efficacy remains contingent upon tissue integrity and mRNA diffusion dynamics [122]. Simultaneously, imaging-based

platforms, such as MERFISH, CosMx Spatial Molecular Imager and Xenium, leverage single-molecule RNA detection to achieve subcellular resolution, albeit within targeted gene panels [123–125]. By resolving the topographical intricacies of CAF-myeloid-lymphoid interactomes, these advanced platforms are converting the CRC TME from a static map into a dynamic blueprint for precision immuno-oncology, paving the way for spatially-defined biomarkers that guide combinatorial therapies. Building on this foundation, the next frontier lies in capturing the temporal evolution of these niches across disease stages. Integrating longitudinal spatial datasets will be essential for linking TME dynamics to clinical resistance, ultimately transforming these atlases into actionable frameworks for patient stratification and the rational design of next-generation CRC therapies.

Supplementary Information

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Supplementary Material 1

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Author contributions
YG conceived the idea of compiling this review. SZ prepared the figures. All authors performed the literature search, wrote the manuscript, and approved the submission of the article.

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Declarations

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