Biology and Preclinical Models of Colorectal Cancer Metastasis

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Abstract

Metastatic colorectal cancer (mCRC) is the principal cause of colorectal cancer (CRC)-related mortality, yet the biology of mCRC remains only partly understood and remains challenging to interrogate experimentally. Despite recent progress in mapping recurrent genetic and epigenetic alterations and treatment responses of mCRC, it provides limited insight into how heterogeneous primary tumors breach tissue barriers, survive in circulation, and colonize distant organs. In this review, we summarize current experimental systems for studying mCRC, including genetically engineered mouse models (GEMMs), carcinogen-induced and transplant models, and patient-derived organoid (PDO) and xenograft platforms, and discuss how each captures or fails to capture key steps of the metastatic cascade and organ-specific microenvironments. We highlight practical obstacles to longitudinal sampling and quantitative readouts of metastatic burden, as well as conceptual gaps in modelling immune and stromal influences. Finally, we outline how emerging approaches, including single-cell and spatial transcriptomics, and advances in longitudinal tracking of metastatic burden could be combined into an integrated framework that more faithfully links mechanistic insight to clinical behavior and ultimately, to metastasis-specific therapies. An overview of the experimental models and integrative technologies discussed in this review is provided in Fig. 1.

Introduction

CRC is among the most common and lethal malignancies worldwide, with an estimated 1.9 million new cases and 900,000 deaths annually.[1] Although early detection and adjuvant therapy have significantly improved outcomes, mCRC remains largely incurable and accounts for nearly 90% of CRC-related mortality.[2] The liver represents the predominant site of metastasis, followed by the lungs and peritoneum, reflecting the portal venous drainage of the colon and rectum.[3] Despite the integration of combination chemotherapy, targeted therapy, and, more recently, immunotherapy, five-year survival for metastatic disease remains below 20%.[4]

Contemporary management of mCRC combines cytotoxic chemotherapy, anti-VEGF and anti-EGFR or anti-BRAF–based targeted regimens, and—where applicable—immune checkpoint inhibitors. However, durable benefit is primarily restricted to molecularly selected subsets of a high level of microsatellite instability/deficiency in mismatch repair (MSI-H/dMMR).

By contrast, most patients with microsatellite stable (MSS) disease derive limited benefit and commonly develops primary or acquired resistance driven by clonal diversity, tumor heterogeneity, cell plasticity, stromal and immune remodeling, and organ-specific microenvironments[5-9]. These realities underscore an urgent clinical need: improving clinical outcomes requires a deeper mechanistic understanding of the metastatic cascade, including initiation, distant organ colonization, and subsequent therapeutic resistance.

Achieving that understanding requires moving beyond descriptive genomics to mechanism—dissecting the sequential steps of dissemination, intravascular survival, extravasation, organotropism, niche conditioning, and immune escape in CRC. However, CRC has been historically difficult to model compared with other solid tumors; many widely used systems capture primary tumorigenesis but incompletely recapitulate spontaneous and reproducible metastatic progression and therapeutic response.[10, 11] Robust, disease-relevant preclinical platforms are therefore essential to translate biological insight into effective interventions for patients.[12] Here, we review the biology of CRC metastasis and the experimental models that support mechanistic and translational studies, highlighting how to align key biological questions with the capabilities and limitations of each system.

Biological programs shaping CRC metastasis

CRC dissemination follows the canonical cascade: local invasion, intravasation, survival in circulation under shear/oxidative stress, arrest/extravasation (liver-first via the portal system), niche adaptation and outgrowth, therapy-conditioned relapse.[13] This trajectory is gated by tumor-intrinsic programs, including aberrant activation of key signaling pathways (WNT/β-catenin, MAPK, PI3K, TGF-β, Notch, Hippo/YAP, and hypoxia/HIFs) together with sequential clonal selection of recurrent driver mutations in canonical CRC oncogenes (*KRAS*, *BRAF*) and tumor suppressor genes (*APC*, *TP53*, *SMAD4*).[13, 14] In the context of genetic alterations, loss-of-function

mutations in *APC*, *TP53*, and *SMAD4* and gain-of-function mutations in *KRAS* and *BRAF* collectively sustain WNT and MAPK activation, promote genomic instability and apoptotic resistance, and subsequently rewire TGF-β from a tumor-suppressive to a pro-metastatic pathway.[14] In parallel, pervasive epigenetic remodeling, including CpG island hypermethylation (CIMP),[15, 16] enhancer/super-enhancer rewiring, alterations in SWI/SNF and histone modifiers (KMT2C/D, SETD2),[17] non-coding RNA regulators (miRNAs/lncRNAs),[15, 18] and alternative splicing[16] enables cell plasticity (epithelial mesenchymal plasticity, secretory/mucinous differentiation),[19] immune evasion,[20] metabolic flexibility,[21] and organ-specific colonization.[22]

Molecular subtypes and mCRC

These tumor-intrinsic (genetic and epigenetic) and -extrinsic (immune cells, stromal cells, extracellular matrix) layers converge on consensus molecular subtypes (CMS),[23] providing a framework for subtype-adapted therapeutic strategies.[24] CMS classification is based on genetic, epigenetic, and transcriptomic data, which reflect distinct biological behaviors and clinical outcomes. CMS is an important tool for personalized medicine in CRC, helping identify which patients are most likely to respond to specific therapies. Briefly, CMS1 (MSI-immune) displays genomic instability with strong immune infiltration; CMS2 (canonical) shows epithelial differentiation with WNT/MYC activation; CMS3 (metabolic) features *KRAS* mutations and metabolic reprogramming; and CMS4 (mesenchymal) exhibits

prominent TGF-β/epithelial mesenchymal transition (EMT) signaling with fibroblast- and angiogenesis-rich stroma.[23] Among CMS, the metastatic landscape is predominantly shaped by CMS2 and CMS4. In metastatic disease, CMS assignments skew toward CMS2 and 4; notably, CMS4 is enriched in liver metastases-associates with poorer prognosis and relative resistance to EGFR-targeted therapy, whereas MSI-h/CMS1 is less frequent but may benefit from PD-L1 blockade.[25-27] Subtype shifts between primary and metastatic sites further underscore plasticity and microenvironmental influence.

However, while CMS offers valuable insights, it is crucial to consider additional molecular subtyping methods. For instance, single-cell RNA (scRNA) sequencing provides higher resolution, capturing more tumor complexity than CMS based on bulk RNA-seq and genomics.[27] Bulk RNA-seq has limitations in capturing the full diversity of the tumor microenvironment. It may not represent the cellular heterogeneity present in tumors, which is essential for understanding metastasis and therapeutic resistance. These limitations in CMS, particularly when based on bulk RNA-seq, must be addressed in future studies, with approaches such as multiomic profiling to provide a more comprehensive view of CRC biology.

Current limitations in studying mCRC

Over the past decade, several reviews have extensively discussed therapeutic strategies, clinical algorithms, and molecular subtypes of CRC.[27] These reviews collectively highlight the genomic complexity and clinical heterogeneity of the

disease, with CMS providing a framework for precision therapy.[23, 27-29] However, despite the genomic and transcriptomic granularity achieved in the clinic, our mechanistic understanding of how CRC spreads and colonizes distant organs remains limited.

Unlike breast or melanoma models,[30, 31] which produce spontaneous and reproducible metastases,[32] CRC models often fail to capture the sequential steps of dissemination, intravascular survival, and colonization.[33, 34] Such gap between descriptive molecular knowledge and functional metastasis biology largely stems from experimental constraints.

For instance, classical *Apc*-mutant mouse models—while most widely used to study intestinal tumor initiation[35, 36]—rarely develop distant metastases and often result in early lethality due to local tumor burden.[37] Similarly, inflammation-associated Azoxymethane (AOM)/Dextran sulfate sodium (DSS) models[38] and multi-allelic combinations such as *Apc; Kras; Trp53* mutations[34, 39] can recapitulate advanced adenocarcinomas and tumor progression under chronic colitis. However, they seldom produce overt distant metastases *in vivo.*[40] This reflects a persistent paradox in CRC research: despite being one of the most genetically well-characterized malignancies, faithfully modeling metastatic dissemination in CRC remains experimentally challenging.[41, 42]

Furthermore, biological features unique to the colon exacerbate these challenges. The complex architecture of the intestinal epithelium, its microbiome-rich

environment, and its dual vascular drainage create a distinct selective landscape for metastatic evolution.[43] The heterogeneity of the tumor microenvironment—ranging from immune-rich right-sided mucinous tumors to fibrotic, TGF-β–driven CMS4 subtypes—likely further limits the reproducibility of preclinical systems.[23, 44] Consequently, most mechanistic insights into CRC metastasis remain inferential, derived from static genomic correlations rather than dynamic in vivo modeling.

Experimental constraints on CRC metastasis research

Although CRC has been extensively modeled at the level of tumor initiation, translating these systems into tractable tools for metastasis research remains challenging. A major limitation stems less from the mere availability of models and more from their restricted temporal and spatial resolution, which makes it difficult to capture how metastatic competence emerges, evolves, and interacts with the host environment in real time.

Intrinsic temporal bottlenecks of in vivo experiments

In vivo metastasis studies face fundamental temporal constraints that limit the ability to capture early dissemination dynamics. Rapid primary tumor expansion frequently triggers premature humane endpoints, reducing the time window in which premetastatic niches, circulating tumor cells, or sub-millimeter

micrometastatic foci can be evaluated in a time-resolved manner.[41, 43] These temporal challenges are further compounded in inducible CRC GEMMs, where the timing and anatomical distribution of tumor initiation depend heavily on the properties of the *CreER^{T2}* drivers used. In CRC GEMMs, conditional knock-out (KO) of tumor suppressor genes (*Apc* or *Trp53*) or expression of oncogenes (e.g., *Kras^{G12D}*), using cell lineage-specific promoters such as *Cdx2-CreER^{T2}* or *Villin-CreER^{T2}*, partly mitigates this issue by enabling tamoxifen-inducible Cre-loxP genetic recombination in the gut epithelium. *Cdx2*-based *CreER^{T2}* drivers preferentially target the distal colon and rectum but show regionally restricted and often incomplete recombination,[45] resulting in heterogeneous tumor initiation.[46] In contrast, *Villin-CreER^{T2}* is active along the intestinal epithelium[47] and shows the strongest expression in small intestinal villus enterocytes with lower levels in the colon. In practice, a *Villin-CreER^{T2}* driver exhibits a leaky (tamoxifen-independent) recombination,[47, 48] and recombination efficiencies vary along the crypt-villus axis, leading to mosaic and asynchronous lesions.[49-51] Other gut-specific Cre drivers, such as *Lgr5-EGFP-IRES-Cre*[52] and *Fabp1-Cre,*[51, 53] provide stem cell- or distal intestine-restricted targeting, respectively, but also introduce regional biases and variability in recombination efficiency. Consequently, while these inducible Cre systems are indispensable for modeling CRC and metastasis in a spatiotemporal manner, they can compromise experimental synchrony and spatial precision, similar to observations made for other tissue-specific *CreER^{T2}* lines.[54]

Spatial restrictions and visualization difficulties

Spatially, the colon's anatomy itself restricts visualization and manipulation. The folded mucosa, crypt architecture, and dual blood supply impede intravital imaging compared with more accessible organs such as the skin or mammary gland.[55] Consequently, even when metastatic dissemination occurs, its earliest stages—local invasion and intravasation—often go unrecorded. Recent advances in two-photon[56,57, 58] and light-sheet microscopy[59] have improved visualization of intestinal tumors, but sustained imaging over weeks remains technically and ethically challenging in live animals.[55, 60]

Immune and stromal context

Another barrier of mCRC preclinical models lies in biological reproducibility. Unlike breast or melanoma models that metastasize in a predictable manner,[61-63] CRC models often display considerable inter-animal variability in tumor burden and metastatic frequency, including in matched-littermate settings where driver genotypes are identical. Differences in inbred background (C57BL/6 vs. FVB/N),[64, 65] sex,[66] and breeding cohort[67] can modulate the penetrance of spontaneous or GEMM-based colorectal tumors and liver metastases, so that *Apc*-driven strains show distinct polyp multiplicity, anatomical distribution, and metastatic propensity.[68, 69] Factors such as microbiome composition, diet, cage environment,

and inflammation further influence tumor behavior.[70] These variables are rarely standardized across laboratories, resulting in inconsistent metastatic frequency and anatomical tropism.

Immune and stromal context of CRC resists reductionist modeling. Subtypes such as CMS1 and CMS4 represent immunologically opposite extremes—one enriched for cytotoxic lymphocytes, the other dominated by fibroinflammatory stroma—yet both can metastasize.[23, 25, 27] Recapitulating these divergent ecosystems requires integrating epithelial, immune, and mesenchymal components within the same experimental system, a feature that remains largely unsolved. Even organoid or patient-derived xenograft (PDX) platforms, while powerful for molecular analysis, fail to fully recapitulate dynamic immune surveillance or the remodeling of premetastatic niches in distant organs.[71-74]

Stem cell hierarchy and plasticity in mCRC

Beyond stromal and immune heterogeneity, the hierarchical organization of CRC adds another layer of complexity to metastasis modeling. Cell lineage-tracing studies have demonstrated that $Lgr5^+$ tumor cells possess the distinct capacity to initiate and sustain distant metastases, whereas $Lgr5^-$ progenitors show limited seeding potential and fail to maintain long-term growth in secondary sites.[75, 76] However, $Lgr5^+$ cells display plasticity, as $Lgr5^-$ populations can reacquire stem-like properties under selective pressure, challenging the concept of a fixed metastatic hierarchy.[76] Current GEMMs and organoid systems capture

aspects of this cell plasticity but still fall short of reproducing its dynamic regulation by the microenvironment.[71, 72]

These challenges partly explain why progress in CRC metastasis research has lagged molecular characterization. They also highlight a conceptual gap: current models allow us to describe *which* genetic and epigenetic events occur, but not *when, where, or under what ecological pressures* metastatic potential arises. Bridging this gap will require longitudinal, multi-scale approaches that integrate imaging, lineage tracing, and omics under physiologically relevant conditions.

Preclinical models

Over the past three decades, multiple experimental platforms have been developed to model CRC and metastasis. Despite substantial progress in capturing genetic diversity and therapeutic responses, these systems rarely reproduce the sequential, spontaneous nature of human metastatic disease. In vivo, CRC cells derived from these platforms are typically introduced into mice through a few standard routes—subcutaneous flank injection, orthotopic implantation into the cecal or rectal wall, and intrasplenic, portal-vein, or tail-vein injection—which in turn determine whether primary tumor growth, liver metastasis, or lung colonization is modeled. Each preclinical model—ranging from cell lines to organoids, PDXs, and GEMMs—offers

complementary insights yet constrained by distinct structural, temporal, and translational limitations that collectively hinder mechanistic discovery.

Cell lines

Cell line-based models remain the most accessible and widely used tools in CRC research.[77] They are inexpensive, easy to propagate and cryopreserve, and highly amenable to genetic manipulation and high-throughput drug screening, and many lines are characterized at the genomic and pharmacologic levels. Human cell lines[78] such as SW480, SW620, and HCT116, together with murine cell lines MC38 and CT26,[79] have provided invaluable insights into oncogenic signaling, drug sensitivity, and epithelial–mesenchymal transition (EMT).[80] SW480 and SW620, derived from primary colon tumors and a lymph-node metastatic carcinoma from the same patient,[78] respectively, offer a convenient paired system to compare molecular features associated with metastatic progression.[81]

In vivo, these cell lines are most frequently used as cell line–derived xenografts. Subcutaneous implantation is the workhorse for tumor growth and drug-response studies, whereas the same lines can be used in orthotopic or intrasplenic/portal-vein models described above to interrogate specific steps of metastatic dissemination.

However, long-term culture often leads to clonal drift, copy-number alterations, and transcriptomic divergence from the parental tumor.[82] Most cell lines represent late-stage or poorly differentiated tumors that have lost the hierarchical organization and cellular heterogeneity characteristic of *in vivo* lesions.[83, 84] Furthermore, monolayer culture lacks stromal and immune components, eliminating the paracrine and mechanical cues essential for invasion and metastasis. Even the frequently cited SW480-SW620 pair captures only a snapshot of metastatic disease and does not recapitulate the dynamic, stepwise evolution of dissemination observed in patients. Thus, while CRC cell lines remain indispensable for reductionist mechanistic studies and scalable pharmacologic screens, their limited architecture and inability to represent full tumor heterogeneity must be carefully considered when extrapolating findings to human disease.

Patient-derived organoids (PDOs)

CRC organoids recapitulate histopathological features and allow genetic manipulation via CRISPR or shRNA,[85] enabling systematic interrogation of key genetic alterations associated with CRC metastasis. Drug screening studies have shown notable concordance between organoid responses and clinical outcomes.[86] Beyond *in vitro* profiling, organoid platforms are also used directly to model mCRC *in vivo*. Orthotopic transplantation of genetically engineered human

or murine CRC organoids into the cecal or rectal mucosa generates primary tumors that can spontaneously seed liver and lung metastases, enabling stepwise analysis of invasion, dissemination, and distant colonization in a controlled genetic and microenvironmental context.[87-89] Portal- or mesenteric-vein injection of organoid-derived cells produces stroma-rich liver lesions that recapitulate the fibroinflammatory niche of human CRC liver metastases and can be used to test stromal or niche-targeted interventions.[90, 91] Syngeneic transplantation of genetically engineered murine organoids into immunocompetent hosts similarly preserves an intact immune system and has been leveraged for *in vivo* CRISPR-based screens to uncover metastasis drivers and therapeutic vulnerabilities[92] (see *'Genetically engineered murine organoids for syngeneic transplantation*' for details).

However, organoids remain inherently reductionistic, lacking the vasculature, fibroblasts, immune cells, and organized extracellular matrix (ECM) organization necessary for invasion and metastasis.[93-96] Assembloids, co-culture systems combining organoids with cancer-associated fibroblasts or lymphocytes, have improved physiological relevance, but reproducibility and scalability are limited.[72, 97, 98] Standardized media formulations, batch effects, and stromal cell sourcing continue to confound inter-laboratory comparisons.[99]

Organoid-on-chip and microfluidic co-cultures

Engineering efforts recently combined PDOs with microfluidic “organ-on-chip” devices to control endothelial cells, shear stress, oxygen, and nutrient gradients. These platforms enable direct observation and quantification of invasion, transendothelial migration, and early steps of dissemination, and they can be extended to drug and immune-response testing as well.[100-102]

In the context of mCRC, these devices have been used to model specific steps of the metastatic cascade. A CRC-on-chip system combining PDOs with perfused endothelial channels reconstructed the colonic mucosa-submucosa interface and enabled live imaging and quantification of invasion and intravasation under defined stromal and flow conditions.[100] Multi-organ “metastasis-on-a-chip” platforms linking a colon tumor compartment seeded with CRC spheroids to downstream liver-like microtissues have been used to study colon-to-liver extravasation, early hepatic outgrowth, and responses to anti-angiogenic or anti-metastatic agents.[103, 104]

Beyond chip devices, 3D microfluidic platforms that co-culture organoids with endothelial cells generate self-organized microvascular networks and visualize tumor–vessel interactions. These proofs-of-concept quantify increased angiogenic sprouting, changes in vascular permeability, and chemotactic coupling between tumor cells and endothelium—key dynamics of the pre-seeding phase of metastasis.[105] Broader syntheses emphasize how flow and shear stress modulate endothelial barriers, angiogenesis, and drug distribution in 3D co-cultures.[102, 106] Despite these advantages, organoid-on-chip and

microfluidic co-culture systems have significant limitations. Matrices and flow regimens are often non-physiologic or poorly standardized, so readouts can shift with lot-to-lot changes in ECM composition, stiffness, shear stress, or oxygen tension.[107] Stromal and endothelial cells frequently lose their phenotypes over time, and adaptive immune cells rarely maintain stable function, restricting studies of immunoediting and immunotherapy.[108-110] Device materials can adsorb hydrophobic drugs and cytokines, while chip-to-chip and donor-to-donor variability, manufacturing cost, and operator dependency hinder scalability and reproducibility.[109, 110] Most platforms also lack lymphatic drainage, innervation, and multi-organ crosstalk.[107] For translational use, careful control and reporting of physical parameters, standardized media/ECM formulations, and side-by-side validation against *in vivo* benchmarks will therefore be essential.[111, 112]

Patient-derived xenografts (PDXs)

PDXs offer higher fidelity in maintaining tissue architecture and inter-patient variability.[99, 113] By implanting patient tumor fragments into immunodeficient mice, PDXs preserve clonal heterogeneity and histological features, making them valuable for drug efficacy and resistance modeling.[114, 115]

In metastasis research, PDXs can recapitulate patient-specific patterns of organotropism and enable evaluation of metastatic outgrowth in a clinically relevant genomic and stromal context.[116, 117] Several studies have shown that

orthotopic or circulation-based PDX implantation can generate spontaneous liver or lung metastases, allowing functional interrogation of metastatic potential and therapy response.[118]

Nevertheless, their dependence on immune-compromised hosts (e.g., Nude or Severe Combined Immunodeficient [Scid] recipient mice) prevents analysis of immune surveillance, tumor-immune crosstalk, and immunotherapy response.[114] Moreover, human and mouse species barriers differ in cytokine signaling, extracellular matrix composition, and microbiome, distorting stromal remodeling and metastatic niche formation.[94, 119, 120] Although PDX models incorporating human immune cells using humanized mice are emerging, they remain technically demanding, expensive, and short-lived due to graft-versus-host reactivity.[113, 121-123]

GEMMs

Early CRC GEMMs, such as *Apc*$^{Min/+}$ mice, recapitulate the classical adenoma-carcinoma sequence in the small intestine but rarely progress to frank invasion or distant metastasis, limiting their utility for metastasis research. To promote malignant progression, conditional alleles of *Apc, Kras*^{G12D} and *Trp53* have been combined with intestine-specific and tamoxifen-inducible Cre drivers (**Table 1**). Upon tamoxifen administration, *Villin-Cre*^{ERT2}*; Apc*$^{fl/fl}$*; Kras*^{G12D} mice generate numerous adenomas throughout the intestinal tract but largely retain a non-

invasive phenotype without macroscopic metastases,[124] whereas *Cdx2-Cre*^{ERT2}-based models restrict recombination to the distal intestine and colon, yielding invasive adenocarcinomas with prominent desmoplastic stroma that more closely resemble human CRC, yet still without significant and consistent distant spread.[125-127]

Further pathway engineering has enabled genuine metastatic behavior in a subset of GEMMs. For example, adding biallelic *Trp53* loss to *Villin-Cre*^{ERT2}*; Apc*$^{fl/fl}$*; Kras*^{G12D} accelerates malignant transformation and produces highly invasive colon tumors with histologically confirmed liver metastases.[39] Similarly, *Fabp1-Cre*-driven deletion of *Apc* and *Tgfbr2* alleles on a *Kras*^{G12D} background yields TGF-β-signaling-deficient carcinomas with desmoplastic stroma, of which 10-20 % give rise to spontaneous liver metastases.[128] These models demonstrate that appropriate combinations of WNT, RAS, p53, and TGF-β pathway alterations drive stepwise progression from adenoma to invasive carcinoma and, in a fraction of animals, clinically relevant hepatic dissemination.

Despite these advances, CRC GEMMs still exhibit several practical limitations. Tumor latency and penetrance are highly variable between strains. Even in "metastatic" models, the frequency and timing of liver lesions remain inconsistent, which complicates adequately powered metastasis studies. Disease progression is also strongly modulated by host-intrinsic variables such as microbiome composition, diet and background inflammation, contributing to substantial inter-animal heterogeneity under nominally identical genotypes.[129-132] Moreover, most

GEMMs develop multifocal primary tumors and early intestinal morbidity that restrict the time window available to interrogate pre-metastatic niches or to impose therapeutic interventions. Thus, while GEMMs provide an immunocompetent setting and faithfully model de novo tumorigenesis, their structural and temporal constraints necessitate complementary platforms—including organoid-based orthotopic and patient-derived xenograft models—to fully dissect the mechanisms of CRC metastasis (**Table 1**).

Genetically engineered murine organoids for syngeneic transplantation

Several groups have recently used tumor organoids derived from the intestine of GEMMs and re-implanted them orthotopically into syngeneic hosts.[87, 88, 133] *Kras*^{G12D} *Trp53* KO murine intestinal organoids, when transplanted into the distal colon, generate locally invasive adenocarcinomas that remain largely confined to the bowel wall, thus providing a technically tractable platform to interrogate invasion in a colon-restricted microenvironment without consistent distant spread.[45, 134] *Apc* KO *Kras*^{G12D} *Trp53* KO intestinal organoids transplanted into the cecum reproducibly form desmoplastic primary tumors and, in a subset of mice, give rise to liver or lung lesions, capturing early metastatic escape in a genetically well-defined setting.[89, 135]

Rationally engineered quadruple-mutant organoids harboring *Apc* KO, *Kras*^{G12D}, and *Trp53* KO along with *Smad4* deletion further increase metastatic efficiency.

TGF-β signaling plays a well-established, context-dependent role in cancer progression:[136] while it restrains epithelial proliferation in early disease, in advanced tumors it is frequently coopted to drive EMT, immune suppression, and metastatic niche formation across multiple cancer types.[137] In CRC, genetic disruption or pathway rewiring of TGF-β/SMAD signaling is associated with poor prognosis,[138] mesenchymal CMS4-like phenotypes, and a higher propensity for liver metastasis.[137, 139, 140] In line with this, organoids derived from *Tgfbr2*$^{fl/fl}$*; Kras*^{G12D}*; Trp53*$^{fl/fl}$ GEMMs, when introduced into the cecal wall or via splenic injection, exploit the portal circulation to establish reproducible liver metastases, highlighting the role of TGF-β signaling loss in invasive behavior and hepatic colonization.[137, 141]

Organoid-based orthotopic models preserve key strengths of GEMMs—tumor growth in an immunocompetent host and within native stromal architecture—while being easier to control experimentally. Defined organoid genotypes and implantation sites allow more synchronized tumor onset, permitting side-by-side imaging and treatment across cohorts. However, engraftment and metastatic yield remain variable, and the immune and microbial environment is still purely murine. These hybrid systems are therefore regarded as a complementary platform rather than a replacement for autochthonous models, well suited to mechanistic studies of the earliest phases of invasion, intravasation, and liver seeding.

Orthotopic transplantation models in PDX/PDO systems

In the clinical translation space, most PDX work has relied on transplantation paradigms based on either subcutaneous or orthotopic engraftment of patient-derived colorectal tumor tissues. In conventional flank xenografts, CRC cell lines or small PDX fragments are implanted under the skin of immunodeficient mice, which makes it easy to monitor and quantify in a non-invasive manner.[113, 142,143] This ectopic setting, however, provides only a rudimentary stromal and vascular niche and therefore offers limited insight into how colorectal tumors invade, disseminate, and colonize distant organs.[37, 143-145] While it does not support spontaneous metastasis, non-invasive bioluminescence imaging (IVIS) can partially compensate for this limitation by enabling longitudinal tracking of tumor burden and early dissemination dynamics.

Orthotopic transplantation protocols instead place PDOs or established CRC cells into the cecum, rectum, or colonic wall of immunocompromised hosts—typically by surgical implantation or intraluminal injection.[146, 147] Tumors arising from these procedures grow along the natural mucosal and vascular axes of the intestine and often reproduce the characteristic pattern of colorectal spread, including liver involvement in a subset of animals.[87, 148-151] In selected CRC orthotopic models, primary cecal or rectal tumors can be surgically debulked or resected to isolate metastatic outgrowth and extend the observational window for liver metastasis, a strategy that has been adopted in a few recent CRC metastasis protocols.[152] However, routine resection of intracecal or intrarectal

primaries is technically demanding, risks disrupting bowel continuity and portal drainage, and can negatively affect animal welfare; consequently, many CRC orthotopic metastasis studies still leave the primary lesions in place and assess metastatic burden in their presence.[153] [154]

Orthotopic PDX/PDO models are better suited than subcutaneous implants for testing site-specific therapies and for mapping the routes by which human CRC cells reach the portal circulation. That said, they remain technically demanding, with engraftment rates and metastatic yield influenced by injection depth, local stromal compatibility, and operator experience.[155, 156] The obligatory use of immunodeficient strains also indicates that adaptive immune surveillance and human-liver crosstalk are only partially captured, so these systems complement rather than replace immunocompetent GEMM-based models in the metastasis toolkit.[157, 158]

Orthotopic co-engraftment (enhanced models)

Recent studies using orthotopic co-engraftment of CRC organoids with patient-matched fibroblasts or endothelial cells report increased metastatic seeding efficiency, underscoring that stromal cues are rate-limiting for successful colonization. A large matched CRC organoid–stroma biobank further showed that co-culture with patient-matched cancer-associated fibroblasts (CAFs) restores stromal/CMS-related programs, improves transcriptional fidelity, and

sharpens functional readouts of drug response and stromal resistance mechanisms.[106] Standardized protocols for simultaneous tumor-plus-stroma orthotopic cecum/rectum implantation enable analysis of growth, invasion, and intravasation, while noting take-rate variability with injection depth, stromal compatibility, and operator experience.[159] In portal-vein models, CRC organoids elicit a fibroblast-rich desmoplastic response that recapitulates human CRC liver metastases stroma, facilitating studies of metastatic seeding and niche-directed therapies.[160] Orthotopic PDXs likewise display spontaneous liver/lung dissemination and reveal associations between metastatic lesions, partial mesenchymal-epithelial transition (MET)/stemness programs, and TGF-β signaling—features well suited for probing the dynamics of dissemination and colonization.[118] Collectively, co-culture/co-engraftment with CAFs and endothelial cells supports a functional view that stromal cues govern metastatic seeding efficiency, linking *in vitro* chips, *ex vivo* microfluidics, and *in vivo* orthotopic/portal-vein systems along one mechanistic continuum.

Longitudinal imaging and metastatic modeling

Despite these advances, longitudinal monitoring of metastatic progression remains challenging because of anatomical inaccessibility and the need for advanced imaging modalities, such as Magnetic Resonance Imaging (MRI), Magnetic Resonance Cholangiopancreatography (MRC), micro-computed

tomography (micro-CT), Positron Emission Tomography (PET), and In Vivo Imaging System (IVIS).[161-165] These modalities have limited sensitivity for detecting sub-millimeter micrometastases, and optical signals are subject to depth-dependent attenuation, which reduces the quantitative accuracy of longitudinal comparisons.[166, 167] Repeated imaging is further constrained by the need for anesthesia or radiation exposure, limiting temporal resolution. Serial sampling of metastatic foci is largely infeasible, preventing direct interrogation of early extravasation, micrometastatic persistence, and early outgrowth stages[167-169].

In addition to orthotopic approaches, experimental metastasis models—notably intrasplenic and portal vein injections—are used to study hepatic colonization. Intrasplenic injection delivers tumor cells into the portal circulation and reproducibly seeds the liver,[170, 171] whereas direct portal vein injection bypasses the spleen and enables tighter control of metastatic burden and timing.[160, 172, 173] These methods provide technically consistent and readily quantifiable information for metastatic kinetics, angiogenesis, and therapeutic responses, while they primarily model later stages of metastasis—circulatory survival and colonization—rather than the early steps of local invasion and dissemination. Longitudinal readouts often require advanced imaging.[172, 174]

Together, orthotopic and experimental metastasis models occupy a critical intermediate position between PDXs and GEMMs. Orthotopic implantation preserves key epithelial–stromal interactions and spontaneous dissemination,

whereas splenic and portal vein injections enable reproducible quantification of hepatic seeding. Yet, all remain constrained using immunodeficient hosts and by incomplete reconstruction of immune and stromal complexity. Integrating these models with advanced imaging, immune-competent backgrounds, or humanized microenvironments will be essential for more physiologically faithful investigation of mCRC.

New technologies and integrative approaches

The recent convergence of single-cell transcriptomics, genomics, spatial transcriptomics, and computational modeling has begun to bridge the long-standing divide between molecular characterization and functional metastasis biology. These technologies provide unprecedented resolution to dissect when, where, and how CRC cells acquire metastatic competence—an aspect that classical experimental systems fail to capture. However, widespread adoption of these emerging platforms remains constrained by high costs, specialized instrumentation and bioinformatics expertise, and limited access to high-quality fresh clinical specimens, which can restrict implementation across institutions.

Single-cell and Spatial transcriptomics: Reconstructing missing dynamics

Single-cell RNA-seq atlases of primary CRC and matched liver metastases have revealed marked epithelial and immune heterogeneity, with distinct metastatic ecosystems that differ from primary tumors.[175, 176] In liver metastases, integrated single-cell and spatial profiling has identified transcriptional programs associated with EMT and invasive behavior, including BHLHE40-driven EMT programs that promote metastatic spread.[177] Single-cell and spatial mapping of CRC liver metastases further charted immune evolution across treatment and unveiled how tumors respond to neoadjuvant chemotherapy.[178] Spatially resolved analyses of CAFs show that CTHRC1^{+} fibroblast subsets act as major sources of *WNT5A*, promote EMT, and are linked to poor prognosis. CAF-immune-epithelial crosstalk is topographically organized within tumors.[179-181] Recent work on the pre-metastatic niche extends these insights, demonstrating that Prok2^{+} neutrophils, tumor-derived small extracellular vesicles, and other systemic cues establish inflammatory and immunosuppressive liver microenvironments that favor CRC seeding.[182,183] Together, single-cell and spatial data give a much more detailed view of which cells and niches drive metastasis than bulk RNA-seq. Nonetheless, single-cell and spatial transcriptomics still miss fragile or deep-lesion cells[184, 185] and are difficult to combine consistently across patients and different platforms.[186]

Multiomics integration

Multiomics studies that combine genomic, transcriptomic, epigenomic, and proteomic data in primary CRC and liver metastases have begun to systematically link recurrent driver alterations with downstream pathway changes.[187, 188] Proteogenomic analyses of matched normal, primary tumor, and liver metastasis triplets integrating whole-exome sequencing, RNA-seq, single-nucleotide polymorphism (SNP) arrays, and quantitative mass spectrometry have identified copy number-mRNA-protein-correlated modules and metastasis-enriched molecules, nominating candidates such as *COL1A2*, *BGN*, *MYH9*, and *CCT6A* with prognostic relevance.[187] In CRC organoids, integrated analysis of the transcriptome, (phospho)proteome, and secretome has shown that *SMAD4* inactivation leads to reduced epithelial differentiation, activation of pro-migratory and proliferative programs, disruption of TGF-β, WNT, and VEGF signaling, and increased secretion of proteins involved in pro-metastatic processes, illustrating how multi-layer measurements map the consequences of a single driver lesion across regulatory levels.[189]

Integrating genomic, transcriptomic, epigenomic, proteomic, and metabolomic data across patients and studies remains technically challenging. Heterogeneous assay performance, missing data, and variation in biospecimen handling, library preparation, and analysis workflows introduce batch effects and other systematic biases. Computational tools such as Harmony, MOFA+, and multimodal Seurat[190-192] help align data from different patients and assays into a shared space and identify common patterns, but batch effects, uneven sampling, and

limited proteomic and metabolomic depth still make metastasis-associated signatures noisy and difficult to reproduce.[193-196]

Computational modeling

Computational modeling has become central for synthesizing these high-dimensional data into mechanistic hypotheses about metastatic behavior. Hu et al. combined spatial tumor growth modeling with statistical inference of matched primary CRC and metastatic exomes to estimate dissemination timing, showing that metastases are frequently seeded early while the primary lesion remains clinically undetectable, thereby challenging a strictly late-stage linear progression model.[197] Using multiregional whole-genome and exome data across primary tumors, multiple metastases, and PDXs, Dang et al. reconstructed clonal relationships and seeding patterns, revealing therapy-shaped evolutionary branching with both mono- and polyclonal dissemination and instances consistent with parallel or metastasis-to-metastasis spread.[198] These reconstructions align with agent-based and multiscale models that simulate clonal competition, spatial constraints, and microenvironmental feedback to generate testable predictions about metastatic outgrowth, recurrence timing, and treatment resistance; West et al. highlighted how these frameworks translate multi-scale data into explicitly mechanistic, hypothesis-driven simulations [199]. Consistent with this view, recent cell lineage-tracing work that couples high-

complexity genetic barcoding with single-cell transcriptomics in esophageal preneoplasia quantitatively maps precursor cell dynamics and lineage plasticity, providing ground-truth constraints for evolutionary models of early neoplastic progression.[144] Dynamical systemic approaches that quantify epithelial mesenchymal plasticity and its association with stemness and immune escape provide a useful framework for interpreting the diverse metastatic cell states observed in single-cell datasets.[200] As multiomic and spatial CRC resources expand, iterative cycles between in silico modeling and in vivo or ex vivo perturbation should increasingly shift metastasis research from retrospective description toward predictive modeling of metastatic fitness landscapes and therapeutic vulnerabilities[199, 201-204].

Despite remarkable advances in molecular profiling and model development, metastasis remains one of the most challenging biological frontiers. The persistent gap between descriptive genomics and functional understanding stems from both biological complexity and experimental constraints. Nevertheless, combining next-generation profiling tools such as single-cell and spatial transcriptomics with innovative model systems including GEMM, organoid models and humanized mice, promises to bridge these long-standing divides. A unified framework integrating temporal, spatial, and molecular dimensions may further illuminate *how colorectal cancer metastasizes—and why it so often resists cure.*

Conclusions and future perspectives

Future progress will depend on constructing a multi-layered ecosystem of experimental and computational approaches. Integrating organoid-based co-cultures, lineage-traced GEMMs, and spatial-omics-guided human tissue analysis can help connect molecular alterations to functional outcomes. Additionally, collaborative metastatic biobanks and standardized computational pipelines will be essential to harmonize preclinical and clinical data across institutions. By merging experimental innovation with computational precision, metastasis can finally be reconstructed as a *dynamic, evolving ecosystem*—one whose vulnerabilities may at last be rendered visible and therapeutically actionable.

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The authors declare no conflict of interest relevant to this article.

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Figure legends

Figure 1. Overview of preclinical platforms and integrative technologies to study mCRC

Patient-derived tumors and genetically engineered mouse models (GEMMs) can be used to generate tumors, cell lines, and organoids. These materials are evaluated using transplantation-based *in vivo* models, including subcutaneous implantation, orthotopic models (cecum/rectum), and intrasplenic/portal-vein injection, as well as microphysiological systems such as organ-on-chip. Across these platforms, advanced analytical approaches—single-cell transcriptomics, genomics, spatial transcriptomics, and computational modeling—enable integrated characterization of metastatic progression and the tumor microenvironment.

Table 1. Representative preclinical models for colorectal cancer metastasis

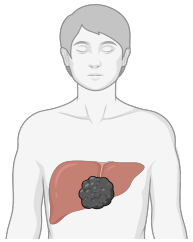
Model type	Representative system	Metastatic route / target	Key features	Ref

GEMMs	*Apc*$^{Min/+}$	No metastasis reported	Classic intestinal tumor model; lacks invasive phenotype	35, 205
	Villin-Cre^{ERT2}; *Apc*$^{fl/fl}$;*Kras*^{LSLG12D}	No distant metastasis observed	Generates multiple intestinal adenomas; non-invasive phenotype	124
	Cdx2-Cre^{ERT2};*Apc*$^{fl/fl}$; *Kras*^{LSLG12D};*Trp53*$^{fl/fl}$	Invasive phenotype without distant metastasis	Colon-specific genetic recombination that reproduces invasive adenocarcinoma with desmoplastic stroma	88
	Villin-Cre^{ERT2}; *Apc*$^{fl/fl}$;*Kras*^{LSLG12D};*Trp53*$^{fl/fl}$	Liver metastasis observed (macroscopic)	Highly invasive adenocarcinomas with confirmed liver metastases	39
	Fabp1-Cre;*Apc*$^{fl/fl}$; *Kras*^{LSLG12D};*Tgfbr2*$^{fl/fl}$	Liver metastases detected in subset (10-20%)	TGFβ-signaling-loss-driven invasion and desmoplasia	128
Orthotopic transplantation of genetically engineered murine organoids	*Cdx2-Cre*^{ERT2};*Apc*$^{fl/fl}$; *Kras*^{LSLG12D};*Trp53*$^{fl/fl}$-derived tumor organoids	Ex vivo organoid culture and orthotopic colonic injection; No distant metastasis observed; localized invasive growth in colon wall	Organoids derived from GEMM generate colon-restricted invasive adenocarcinomas upon orthotopic transplantation; faithfully mimic human CRC architecture and desmoplastic stroma but lack metastatic spread	88
	Villin-Cre^{ERT2}; *Apc*$^{fl/fl}$;*Kras*^{LSLG12D};*Trp53*$^{fl/R172H}$-derived tumor organoids	Orthotopic transplantation into cecum wall; occasional metastasis to liver and lung		87, 206
	Apc$^{-/-}$;*Kras*$^{LSLG12D/+}$;*Trp53*$^{-/-}$; *Smad4*$^{-/-}$	Orthotopic transplantation of genetically engineered intestinal or colonic organoids into cecum/rectum; metastasis to liver and lung	Reproducible macroscopic metastases; recapitulates adenoma-carcinoma-metastasis sequence	87, 89, 207
	Apc$^{fl/fl}$; *Kras*^{LSLG12D}; *Tgfbr2*$^{fl/fl}$;*Trp53*$^{fl/fl}$ GEMM-derived organoids	Orthotopic cecal or splenic injection of *in vitro* Ad-Cre–recombined tumor organoids; portal dissemination to liver (occasional lung lesions)	TGFb-signaling loss drives invasive adenocarcinoma and reproducible liver metastasis	137
Orthotopic transplantation of PDOs	Human CRC PDOs	Subcutaneous and orthotopic into cecal or rectal wall. No distant metastasis reported.	Human PDOs maintain histological and genetic fidelity to the parental tumor; first demonstration of *in vivo* tumorigenicity of human CRC organoids	208
		Orthotopic portal vein injection; metastasis to liver	Human PDOs reproducibly form hepatic metastatic nodules following portal vein injection; faithfully mimic desmoplastic and fibroblast-rich stroma observed in clinical CRC liver metastases	90
	Human CRC Primary- and Metastatic-derived PDOs	Subcutaneous and orthotopic into cecal or rectal wall; occasional metastasis to liver	Occasional liver metastases observed only in mice transplanted with metastatic-origin PDOs; none in primary PDO group.	209
Non-orthotopic transplantation of murine cancer cell lines	CT26 (BALB/c)	Tail vein injection; metastasis to lung	Formation of lung metastases within ~2 weeks after injection	210
		Intrasplenic injection or intraportal; metastasis to liver	Mimics hematogenous spread via portal circulation; widely used for hepatic metastasis evaluation	34, 211
	MC38 (C57BL/6)	Intrasplenic or intraportal vein injection; metastasis to liver and occasionally to lung	Highly reproducible hepatic metastasis via portal circulation; mimics hematogenous spread under immunocompetent background. Preferred routes for MC38 due to low orthotopic engraftment efficiency.	34, 156, 171, 211
Non-orthotopic transplantation of	KM20L2, HCT116, HCT15, SW480, SW620, Colo320DM		SW620: 20% liver metastasis;	212

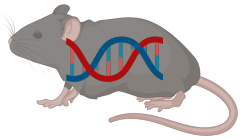
human CRC cell lines		Orthotopic cecal injection; occasional metastasis to liver and lymph nodes	Common nodal metastasis except for SW480, Colo320DM	
	Co115		Tumor take rate 90%; metastasis to nodal and occasionally to liver	212
	HCC2998		Tumor take rate 88%; metastasis to nodal and rarely to liver	212
	HT29		Tumor take rate 69%; metastasis to nodal and rarely to liver	212
	CaCo2, WiDr, Co205		Tumor take rate 40%; very low metastasis	212
	HCT116	Orthotopic cecal submucosa (micropipette injection)	Tumor take rate 75%; metastasis to 100% nodal, 67% liver, and 50% lung	213
		Rectal wall (rectal injection)	Tumor take rate 65%; rare metastasis (3.3%)	214
		Intraportal injection	90% developed liver metastasis (the highest hepatic take rate) within 30 days	215
	HT29	Intrasplenic injection (metastasis to liver)	78% developed macroscopic liver metastasis within 6 weeks	216
	SW620	Intrasplenic injection (metastasis to liver)	~80% liver metastasis within 4-6 weeks	155
		Intraportal injection (metastasis to liver)	100% liver metastasis in all injected mice (dose-dependent tumor load)	38

GEMMs: Genetically engineered mouse models; Min: Multiple intestinal neoplasia; Cre: Cre recombinase; Cre^{ERT2}: Cre recombinase fused with estrogen receptor (ER) conditionally activated by tamoxifen (T2); fl: floxed (flanked by loxP sites, conditionally deleted by Cre recombinase); LSL: a loxP-stop-loxP cassette conditionally removed by Cre recombinase for subsequent expression of gene(s); PDOs: patient-derived organoids; tumor take rate (%) = number of animals developing tumors / total number of animals inoculated/transplanted.

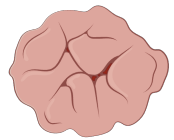
Patients



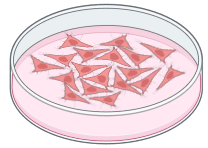
Genetically engineered mice



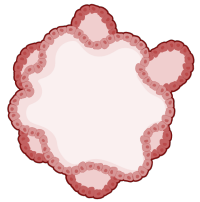
Tumor



Cell line



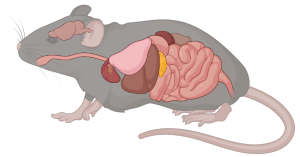
Organoids



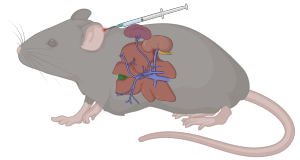
Transplantation

Subcutaneous

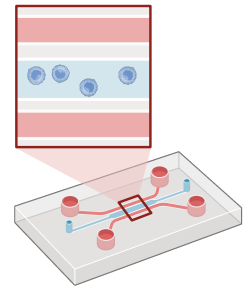
Orthotopic (cecum, rectum)



Intrasplenic/portal vein

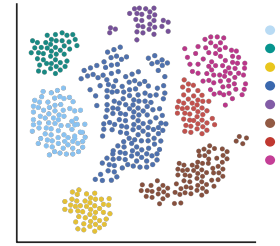


Organ-on-chip

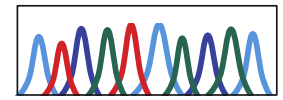


Advanced technologies

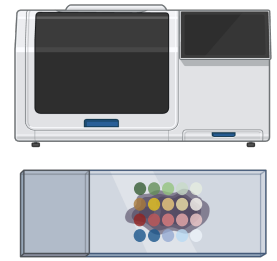
Single-cell transcriptomics



Genomics



Spatial transcriptomics



Computational modeling