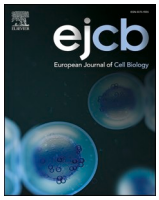
European Journal of Cell Biology 104 (2025) 151478

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.com/locate/ejcb



Mouse colorectal cancer organoids: Lessons from syngeneic and orthotopic transplantation systems

Yu Muta, Yuki Nakanishi *

Department of Gastroenterology and Hepatology, Kyoto University Graduate School of Medicine, Kyoto, Japan

ARTICLE INFO

Keywords:
Colorectal cancer
Cancer organoids
Orthotopic transplantation
Syngeneic transplantation
Tumor microenvironment
Tumor immunity

ABSTRACT

Colorectal cancer (CRC) organoids provide more accurate and tissue-relevant models compared to conventional two-dimensional cultured cell cultures. Mouse CRC organoids, in particular, offer unique advantages over their human counterparts, as they can be transplanted into immunocompetent mice. These syngeneic transplantation models create a robust system for studying cancer biology in the immunocompetent tumor microenvironment (TME). This article discusses the development and applications of these organoid systems, emphasizing their capacity to faithfully recapitulate *in vivo* tumor progression, metastasis, and the immune landscape.

1. Introduction

Colorectal cancer (CRC) is the third most common malignancy globally, with growing incidence and mortality (Bray et al., 2024). CRC is a complex and heterogeneous disease that can be stratified into different subsets based on anatomical, histopathological, and transcriptomic features that are closely related to each other (Ciardiello et al., 2022; Dekker et al., 2019). Accumulating evidence regarding CRC tumorigenesis and progression has led to the identification of molecules and pathways that can be therapeutically targeted in certain types of CRCs. These targets include VEGF, EGFR, BRAF, and HER2, and monoclonal antibodies or tyrosine kinase inhibitors against them are now widely used in the clinic (Shin et al., 2023). Another emerging class of cancer therapeutics is immune checkpoint inhibitors (ICIs), which are designed to reprogram T cell exhaustion in the tumor microenvironment (TME). Monoclonal antibodies against PD-1, PD-L1, and CTLA-4 have been applied to clinical use, and now they are approved for more than 50 cancer types, serving as key drugs in their therapy(de Miguel and Calvo, 2020). Nonetheless, only a small subset of CRC patients can benefit from ICI therapy, as the majority of CRCs are immune suppressive and resistant to ICIs (Weng et al., 2022). Thus, ICIs are effective for specific CRC subtypes that show a hypermutant phenotype, termed microsatellite instability high (MSI-H) status, caused by the inactivation or deficiency of mismatch repair genes (dMMR) (Le et al., 2017; Le et al., 2015). Although ICIs are highly effective for MSI-H/dMMR CRCs (André et al., 2020; Cercek et al., 2022; Lenz et al., 2022; Overman et al., 2018), these subtypes account for approximately 15 % of overall CRCs (Jenkins et al., 2007). Therefore, there is an urgent need for an in-depth understanding of the TME landscape whereby CRCs acquire immune surveillance evasion and therapeutic resistance, as well as an adequate model system to study the TME.

The TME comprises a wide variety of non-cancer cell types, including lymphocytes, myeloid cells, cancer-associated fibroblasts (CAFs), endothelial cells (ECs), pericytes, and adipocytes. This complex collection of cells is embedded in various densities of non-cellular extracellular matrix (ECM) components, depending on the cancer type, which provides structural support, a reservoir for secreted proteins, and scaffolds for cell adhesion. The roles of individual cell types can have either tumor-suppressive or tumor-promoting phenotypes, depending on the context, driven by extensive reciprocal crosstalk through direct cell-cell contact or paracrine secretion of cytokines, chemokines, and growth factors (de Visser and Joyce, 2023). In addition to these classical mediators, other classes of messengers, such as non-structural matricellular proteins (MCP) and extracellular vesicles (EVs), are also secreted to convey the intercellular signals (Gerarduzzi et al., 2020; Lucotti et al., 2022b). Importantly, these messengers not only modify the local environment, but also exert systemic effects far beyond the primary tumor site, such as macrophage recruitment from the bone marrow or T cell suppression in the lymph nodes (Chen et al., 2018; Lucotti et al., 2022a; Peinado et al., 2012; Poggio et al., 2019). Hence, experimental systems are essential that can fully recapitulate both the local tumor environment and the inter-organ networks at the same time.

* Corresponding author.
E-mail address: yuki@kuhp.kyoto-u.ac.jp (Y. Nakanishi).

https://doi.org/10.1016/j.ejcb.2025.151478
Received 19 August 2024; Received in revised form 1 January 2025; Accepted 4 February 2025
Available online 6 February 2025

In this context, mouse CRC organoid models combined with syngeneic transplantation into immunocompetent mice offer significant advantages in the matrix of experimental systems and the species of origin (Table 1). As for *in vitro* experimental systems, organoids serve as more accurate and tissue-relevant models compared to conventional two-dimensional cultured cell lines. Cultured cancer cell lines have been used indispensably, particularly in the fields of biochemical research and large-scale screening. However, it is still difficult to stably create cancer lines with high efficiency and quality as primary cell lines (Shoemaker, 2006). This is partly because they require such features as uniform monolayer growth and attachment to culture plates, which are not necessarily relevant to the tumor progression *in vivo*. Therefore, this establishment process creates a strong selection pressure that biases the cellular behaviors. In fact, when applied to transplantation models, cancer cell lines often exhibit poorly differentiated histology lacking the pathological variations, such as cellular and tissue heterogeneity and structural abnormalities observed in human cancer tissues (Masters, 2000). In contrast, CRC organoids can be easily generated without any additional transformation, regardless of the species. Regarding the species of origin, mouse CRC organoids have fewer ethical concerns compared to human organoids. In addition, they allow syngeneic transplantation into immunocompetent mice, enabling the study of cancer biology, while maintaining the appropriate constitution of the TME, which is technically challenging in human xenograft models. While mouse cancer cell lines are also applicable for syngeneic transplantation, commonly used mouse cancer cell lines, such as CT26 and MC38, do not precisely reflect the CRC carcinogenesis process because they are both generated by induction with exogenous carcinogens (Corbett et al., 1975; Wang et al., 1995). Finally, genetically modified mouse models, represented by Apc^{Min} mice (Moser et al., 1990), the first mouse model of CRC, remain the mainstay of *in vivo* disease models, as they reconstitute the full components of the TME (Kucherlapati, 2023). Endogenous tumor development and well-defined gene mutational profiles enable the demonstration of a relatively direct cause-and-effect relationship. Nevertheless, multiple gene mutations are necessary for advanced-stage CRC models with metastatic potential, which require substantial time and effort for breeding and crossing.

Collectively, to study the biology of cancer cells and the surrounding TME, mouse CRC organoids can serve as central hubs for various experimental systems and resources, including CRC cell lines, human CRC organoids, and genetically modified mouse models. This review outlines the diverse applications of mouse CRC organoids, with a focus on the syngeneic orthotopic transplantation system, highlighting key insights and results obtained from this system.

Table 1
In vitro and in vivo disease models for CRCs.

Model type	Advantages	Disadvantages	Relevance to TME and tumor immunity
Cultured cell lines	Widely used High throughput Easy handling and cost-effective	Low establishment efficiency Limited cell lines for mouse CRCs	Lacks tissue heterogeneity No immune system reaction with human cells
Mouse CRC organoids	Genetically engineered Derived from genetically modified mice Requires no transformation	Less variation than PDOs No models for SSL/SSLDs	Syngeneic transplantation into immunocompetent mice Full recapitulation of the immune landscape
Human CRC organoids	Genetically engineered Derived from patient samples (PDOs) Requires no transformation	Ethical concerns Requires immunocompromised mice for transplantation	Partial recapitulation of the immune landscape when co-transplanted with immune cells
Patient-derived xenografts (PDX)	Generated from clinical samples or PDOs Contains stromal cells	Requires immunocompromised mice High cost and effort.	No immune system interaction
Humanized mice	Recapitulates human immune system	Requires immunocompromised mice High cost and time-consuming Requires HSC engraftment	Partial recapitulation of the immune landscape.
Genetically modified mouse models	Endogenous tumor development Well-defined gene mutation profiles Direct cause-and-effect relationship	Complex breeding and crossing Difficult to model advanced CRCs	Full recapitulation of the immune landscape

2. Development of organoid systems

The intestinal epithelium is the most rapidly cycling tissue in the mammalian organs, renewing every 4–5 days (van der Flier and Clevers, 2009). This rapid turnover is maintained by the Lgr5-positive intestinal stem cells located at the crypt bottom, which generate multiple lineages, including enterocytes, goblet cells, neuroendocrine cells, Tuft cells, and Paneth cells (Barker et al., 2007). This complexity has hindered the maintenance of untransformed intestinal epithelial cells *in vitro*, until the organoid culture systems were developed by two groups in the late 2000s (Ootani et al., 2009; Sato et al., 2009). In Sato's model, purified Lgr5-positive intestinal epithelial stem cells were embedded in Matrigel and cultured in serum-free media supplemented with stem cell niche factors, including EGF, noggin, and R Spondin-1 (Sato et al., 2009). As stem cells supply terminally differentiated progenitors, organoids create budding structures that mimic the crypt-villous architecture observed in the original tissue histology. Another method developed by Kuo and colleagues utilizes intestinal fragments that include stromal components. The fragments were embedded in collagen type I gel and maintained under specific culture conditions known as air-liquid interface (ALI) (Ootani et al., 2009). While these methods aim to establish organoids from adult intestinal tissues and stem cells, alternative approaches have been developed using embryonic stem (ES) cells or induced pluripotent stem (iPS) cells, which replicate the differentiation of embryonic towards the endoderm, hindgut, and intestines (Spence et al., 2011). In any case, these organoid culture systems enable intestinal stem cells to self-renew and produce their progenitors in a three-dimensional structure. Furthermore, by fulfilling tissue specific niche factor requirements, organoids can be established not only from the gastrointestinal tract but also from a variety of organs, including the liver, pancreas, mammary glands, and skin (Boonekamp et al., 2019; Huch et al., 2013a; Huch et al., 2013b; Zhang et al., 2017). Taken together, the organoid system has opened a new avenue for an independent long-term *in vitro* culture system that recapitulates pathophysiological activities in the tissues of origin more accurately than the classical two-dimensional cultured cells.

3. Human CRC organoids: their potential and limitations

While organoids were initially created from mouse tissues, modification of the culture media also facilitates the growth of organoids derived from human normal and tumor tissues (Lo et al., 2020). In case of human colon organoids, a TGF-β inhibitor A73–01 and a p38 inhibitor SB202190 are the niche factors additionally required to sustain their growth (Sato et al., 2011). Growth factor screening further identified a combination of IGF-1 and FGF2, which allows human colon organoids to be efficiently established from a limited amount of tissues such as

endoscopic biopsy samples (Ding et al., 2022; Fujii et al., 2018). Human CRC organoids can be created either by sequential induction of CRC driver gene mutations into normal colon organoids in a "bottom-up" manner, mimicking the adenoma-carcinoma sequence (Drost et al., 2015; Matano et al., 2015), or directly from clinical patient samples as patient-derived organoids (PDOs) in a "top-down" manner (Schutgens and Clevers, 2020). These human CRC organoids overcome the problem of limited access to human clinical samples and have great potential for a variety of downstream applications, including disease modeling, drug screening, and biobanking (Yoshida, 2020). Human CRC organoids can also be utilized to study the interactions between cancer and stromal cells such, as cancer-associated fibroblasts (CAF) and immune cells, using *in vitro* co-culture systems or *in vivo* xenograft models (Dijkstra et al., 2018; Fujii and Sato, 2021; Strating et al., 2023; Subtil et al., 2023; Yoshida, 2020).

In addition to the advantages over conventional two-dimensional cancer cell lines such as better recapitulation of the original tumors, the key strength of PDOs is their capacity to capture the breadth of genetic and molecular diversity observed across different patients. Efficient derivation directly from individual CRC patients allows for the assessment of driver gene mutations, epigenetic alterations, and cellular behaviors on a patient-by-patient basis, all of which strongly impact clinical outcomes. Thus, patient-derived CRC organoids can serve as a personalized platform for biomarker discovery, predicting treatment response or prognosis (Chalabi et al., 2020; Ganesh et al., 2019; Herpers et al., 2022; Kong et al., 2020; Ooft et al., 2019; Park et al., 2021; Toshimitsu et al., 2022; Yao et al., 2020). Reflecting this capacity for individualized care, several studies have reported the usefulness of PDOs in guiding treatment strategies in CRC patients (Mauri et al., 2021; Ooft et al., 2021). Taken together, the PDO system has opened a new avenue for translational applications, leading to tailor-made precision medicine.

Despite their unique advantages, human CRC organoids have significant limitations, particularly in terms of tumor immunity. As immunological responses involve multiple cell types, the human CRC organoid system cannot fully reconstitute the complex immune landscape *in vitro*, even with a co-culture system. In fact, co-culture with macrophages only partially restored the transcriptomic profile of established PDOs, which were altered from the original CRCs (Li et al., 2023). Since ALIs possess TME components, including immune cells, patient-derived ALIs have been reported to recapitulate the ICI response. However, these stromal cells are only transiently present upon generation, as they gradually decay within two months (Neal et al., 2018).

Implantation of surgically dissected human cancer samples has enabled the establishment of patient-derived xenograft (PDX) models. Since PDX models include full layers of cancer tissues, they display histological features similar to those of the original tissues and have the potential to predict treatment efficacy (Yoshida, 2020). PDOs can also be used as grafts to establish PDX in a similar fashion; however, they also require highly immunocompromised mice, such as NOD, NOG, or NSG mice, to achieve a reasonable engraftment success rate (Yoshida, 2020). In these mice, most immune cell types, including T cells and B cells, are absent, making it virtually impossible to investigate tumor immunity (Hesselton et al., 1995; Ito et al., 2002; Makino et al., 1980; Shultz et al., 2005). Recent advances in humanized mouse models have addressed this issue. Humanized mouse models involve immunodeficient mice co-engrafted with human tumors and immune cells. They can be generated by injecting mature human peripheral blood mononuclear cells (PBMC) or human hematopoietic stem cells following sublethal irradiation (Chuprin et al., 2023). While there are pros and cons for each method, the establishment of humanized mouse models requires substantial cost, time, and effort, and no method thus far can fully reconstitute the complex human immune landscape in a mouse model (Bareham et al., 2021). In this regard, mouse CRC organoids can play a crucial and unique role distinct from that of human CRC organoids.

4. Syngeneic and orthotopic transplantation with mouse CRC organoids

Several groups have independently established syngeneic and orthotopic transplantation systems using mouse CRC organoids bearing driver gene mutations, such as those in *Apc*, *Kras*, and *Trp53*, to reconstitute the adenoma-carcinoma sequence (Table 2, Fig. 1) (de Sousa e Melo et al., 2017; Felchle et al., 2024; Fumagalli et al., 2017; O'Rourke et al., 2017; Roper et al., 2017; Tauriello et al., 2018; Varga et al., 2020). Organoids are typically generated from tumors that develop in genetically modified mice harboring these mutations. Alternatively, the CRISPR-CAS9 genome editing technique is also a feasible option for mutating one or more genes in the cascade (Geurts and Clevers, 2023). Additionally, drug-inducible systems using tamoxifen or doxycycline can control the precise timing of gene mutation, overexpression, or silencing (Cheung et al., 2020; Dow et al., 2015; O'Rourke et al., 2017; Roper et al., 2017; Sacchetti et al., 2021; Tauriello et al., 2018). Thus, oncogenic alterations can be induced *in vivo* before tumors are harvested, *in vitro* after organoids are established, and even after the organoids are transplanted. Upon application in *in vivo* cancer models, these mouse CRC organoids can be transplanted into immunocompetent mice of the syngeneic strain. The anatomical sites for transplantation are of great importance. Traditionally, subcutaneous tumor models have been widely used because of their technical feasibility for setup and monitoring. However, subcutaneous tumors exhibit distinct vasculature, ECM composition, and immune landscape from those of the original cancers, all of which hamper the study of *in situ* tumor progression and treatment efficacy (Stribbling et al., 2024). Therefore, the orthotopic transplantation model is a more suitable approach to fully recapitulate the complex TME in human cancers.

From a technical perspective, organoids are dissociated, resuspended in Matrigel-containing media, and injected into the rectal mucosa under direct vision or colonoscopy guidance (Fig. 2) (de Sousa et al., 2020; Roper et al., 2018). Short-term treatment with dextran sodium sulfate (DSS) prior to injection has been reported to improve the engraftment rate in some models (O'Rourke et al., 2017; Varga et al., 2020; Yui et al., 2012); however, mucosal injury and subsequent inflammation might confound the local environment. Another suitable site for orthotopic transplantation is the serosal side of the cecal wall (Cañellas-Socias et al., 2022; Cheung et al., 2020; Fu et al., 1991; Fumagalli et al., 2017; Fumagalli et al., 2020; Fumagalli et al., 2018; Ganesh et al., 2020; Sacchetti et al., 2021; Tauriello et al., 2018). Although laparotomy is required, the large peritoneal cavity allows tumors to grow without causing intestinal obstruction, which increases the chance of metastasis. Furthermore, as engrafted cecal tumors can be surgically removed, this model can be a powerful tool to observe postoperative metastatic recurrence, which is experienced by one-third of CRC patients after curative resection (Nors et al., 2024). Along with single-cell RNA sequencing (scRNA-seq) analyses, the orthotopic recurrence model identified a tumor subpopulation named HRCs with a high potential to relapse after resection. Notably, these HRCs lack a mature TME and are prone to an immune response in the early stages, suggesting that neoadjuvant immunotherapy is a new treatment strategy for preventing postsurgical relapse (Cañellas-Socias et al., 2022). Whereas orthotopically implanted mouse CRC organoids eventually develop metastasis to distant organs and lymph nodes, rapid metastasis can also be achieved using other injection models (Fig. 2). Intrasplenic, intrahepatic, and portal venous injections are effective methods for liver metastasis, while hydrodynamic tail venous injections are used for lung and/or liver metastasis (de Sousa e Melo et al., 2017; O'Rourke et al., 2017; Sakai et al., 2018; Taraborrelli et al., 2023). A comparison between orthotopic and metastatic models revealed differential dependency on Lgr5-positive stem cells in primary and metastatic cancer cells (de Sousa e Melo et al., 2017), which was further supported by the observation that Lgr5-negative cells were the main source of distant metastasis (Fumagalli et al., 2020). More importantly, these systems in

Table 2
Orthotopic transplantation models with mouse CRC organoids.

Driver gene mutations/alterations in engrafted mouse CRC organoids	Host mouse genotypes	Transplantation site	Pretreatment	References
Apc, Kras, Trp53 *Apc, Kras, Trp53, SOX17*	Wild-type	Rectal mucosa	None	(Goto et al., 2024; Roper et al., 2018, 2017)
Apc, Kras, Trp53, Tgfbr2	Wild-type	Cecal wall, portal vein	None	(Cañellas-Socias et al., 2022; Tauriello et al., 2018)
Apc, Kras, Trp53, Smad4	Wild-type, nude mice	Colon mucosa, tail vein, splenic vein, liver	DSS	(O'Rourke et al., 2017)
Apc, Kras, Trp53, Smad4	Wild-type	Rectal mucosa, portal vein	None	(de Sousa et al., 2020; de Sousa e Melo et al., 2017)
Apc, Kras, Trp53, Tgfbr2, Akt	Wild-type	Colon mucosa	DSS	(Varga et al., 2020)
Apc, Kras, Trp53, Smad4	NSG	Cecal wall, portal vein	None	(Fumagalli et al., 2017, 2020, 2018)
Ctnnb1, Kras, Cdkn2a	Wild-type	Rectal mucosa	None	(Felchle et al., 2024)
Apc, Kras, Trp53	NSG	Cecal wall	None	(Sacchetti et al., 2021)
Apc, Kras, Trp53, Yap	NSG	Cecal wall, spleen	None	(Cheung et al., 2020)
Apc, Kras, Trp53, L1cam	NSG nude mice	Cecal wall	None	(Ganesh et al., 2020)
Apc, Kras, Trp53, Tgfbr2, Fbxw7	Wild-type, NSG	Spleen	None	(Sakai et al., 2018)
Apc, Kras, Trp53, Smad4, Atg16l1	Wild-type, NSG	Rectal mucosa, tail vein	None	(Taraborrelli et al., 2023)
Apc, Kras, Trp53, Smad4	Wild-type	Rectal mucosa	None	(Westcott et al., 2021)
Apc, Kras, Trp53, Tgfbr1 *Kras, Trp53, Notch1*	Wild-type, nude mice	Rectal mucosa, spleen	None	(Jackstadt et al., 2019)
Apc, Kras, Trp53, Tgfbr2	*Prkczf/f;Fsp1-Cre, Prkczf/f,*	Rectal mucosa	None	(Kasashima et al., 2021a, 2021b)
Apc, Kras, Trp53, Tgfbr2	Wild-type, *Thbs1-/-, Cd36-/-, Cd47-/-, Thbs1f/f;LysM-Cre, Rosa26-EYFP;LysM-Cre*	Rectal mucosa, spleen	None	(Omatsu et al., 2023)
Apc, Kras, Trp53, Tgfbr2	*Ppp3r1fl/fl;LysM-Cre, Ppp3r1fl/fl*	Rectal mucosa	None	(Peuker et al., 2022)
Apc, Kras, Trp53, Tgfbr2, Prkci, Prkcz	Wild-type	Spleen	None	(Martinez-Ordoñez et al., 2023)

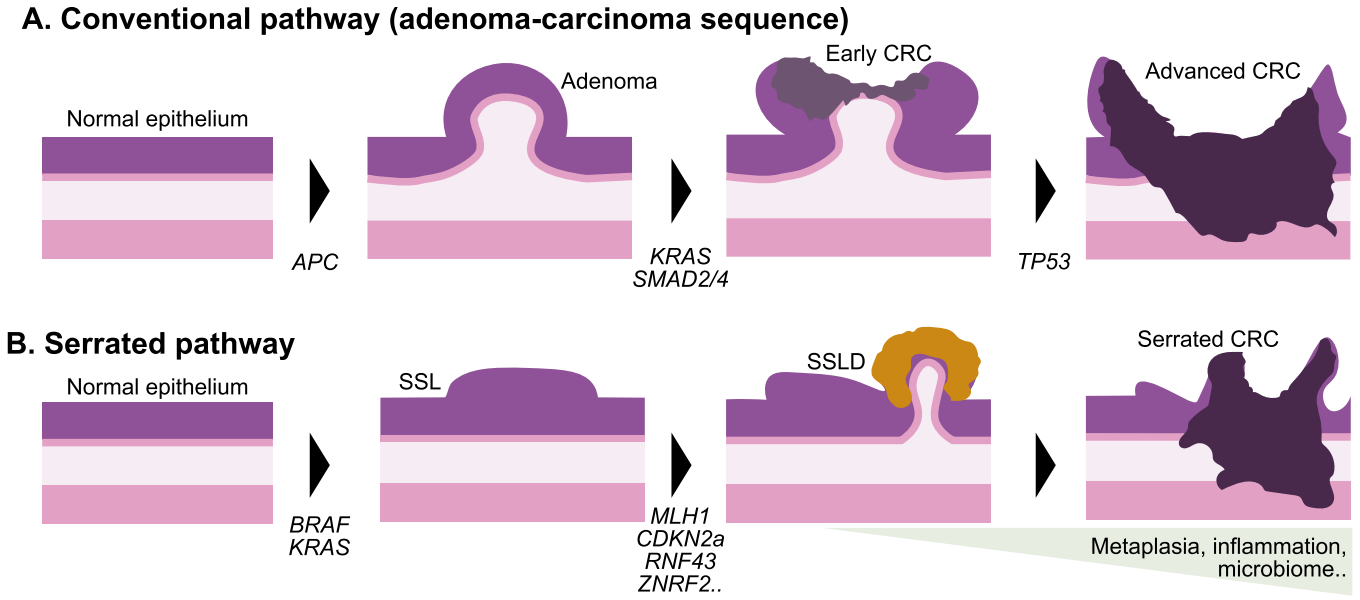


Fig. 1. Progression model of colorectal cancer. Schematic of conventional pathway (adenoma-carcinoma sequence) (A) and serrated pathway (B). (A) The initial step of the sequence is typically *APC* mutation. As additional driver gene mutations accumulate in the tumors, they gain malignant potential in a stepwise manner. (B) In contrast to conventional pathway, *KRAS* or *BRAF* is often mutated in serrated pathway. Precise mechanisms remain to be fully elucidated how SSLs advance to SSLDs and CRCs, although several genes as well as metaplasia, inflammation, and microbiota are reported to be involved in the process.

immunocompetent host mice revealed how tumor intrinsic signaling pathways affect the surrounding immune landscape. For instance, additional mutations in members of the TGFβ signaling pathway, such as *Tgfbr2* or *Smad4*, were shown to promote tumor growth and metastatic capacity (de Sousa e Melo et al., 2017; Fumagalli et al., 2017; O'Rourke et al., 2017; Sakai et al., 2018; Tauriello et al., 2018), which is in good concordance with the observation of the xenograft models using human engineered CRC organoids (Drost et al., 2015; Matano et al., 2015). In addition, tumors exhibited desmoplastic changes, T cell exclusion, and low neoantigen expression, thereby causing T-cell dysfunction and

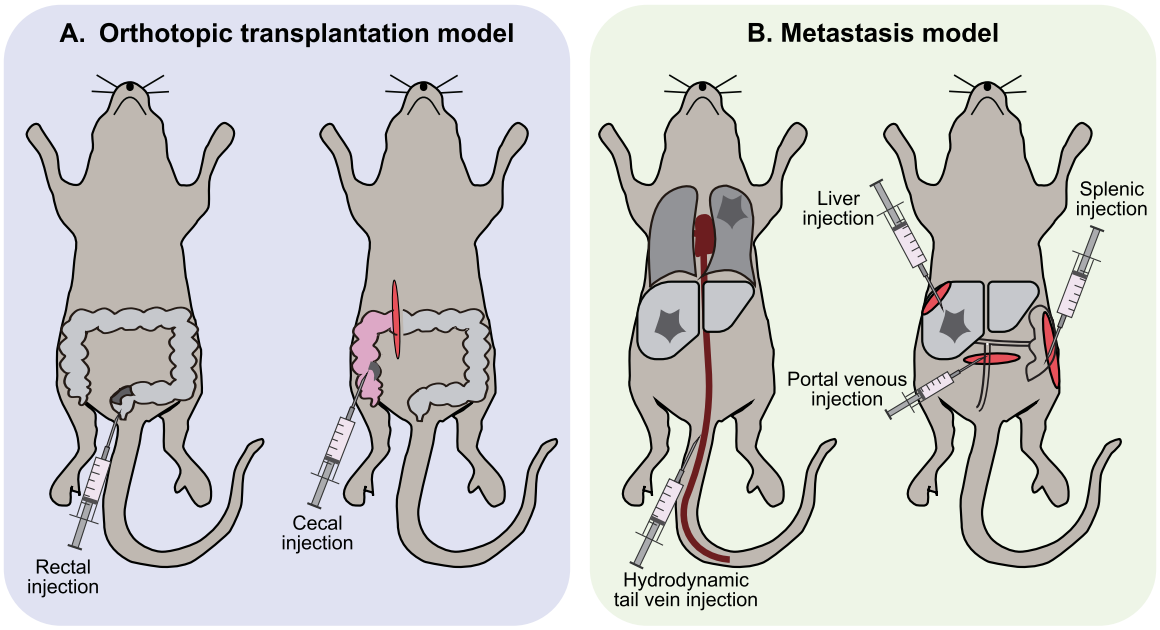


Fig. 2. Orthotopic and metastatic injection model for colorectal cancer. Schematic depicting anatomical sites for orthotopic transplantation models (A) and metastasis models (B). (A) While rectal injection is performed under direct vision or endoscopy assistance, cecal injection requires laparotomy. (B) Hydrodynamic tail vein injection models are used for liver and/or lung metastasis. Direct liver injection, portal venous injection, and splenic injection models are for liver metastasis, which also require laparotomy.

resistance to ICI treatment (Tauriello et al., 2018; Westcott et al., 2021). A similar immunosuppressive phenotype was observed when NOTCH signaling was upregulated by constitutively active *Akt* or *Notch1* (Jackstadt et al., 2019; Varga et al., 2020). In contrast, the loss of Atg16L1, a core component of the autophagy elongation system, enhanced IFN signaling and PD-1 expression, thereby driving the tumor immune response in the orthotopic hydrodynamic tail vein injection model (Taraborrelli et al., 2023).

Of note, the invasive capacity differs among studies, even when they share the same gene mutational profiles. This is partly because sequential transplantation induces transcriptomic and epigenetic alterations that allow tumors to evolve and adapt to the TME(Goto et al., 2024). By comparing the transcriptomic and epigenetic profiles of mouse CRC organoids before and after orthotopic transplantation, Goto and colleagues identified that the transcription factor Sox17 is highly expressed in transplanted organoids. Subsequent orthotopic transplantation experiments with Sox17-knockout organoids further demonstrated that Sox17 orchestrates the immune-evasive phenotype by reprogramming the IFNγ response and MHC-I expression (Goto et al., 2024). Collectively, these studies highlight the vital and unique advantages of syngeneic mouse CRC organoid transplantation models, particularly in tumor immunity research.

5. Genetically modified mouse models as syngeneic hosts

Since mouse tumor organoids can be readily transplanted into mice in a syngeneic fashion, we can leverage the extensive catalog of genetically modified mouse models when choosing transplantation host mice (Fig. 3). By creating cell-type specific conditional knockout mice using site-specific recombination technology, such as Cre-LoxP or Flt-FRT systems (Branda and Dymecki, 2004), we can observe how the "genes-of-interest" are related to tumorigenesis in the context of individual components of the TME.

For example, orthotopic implantations of mouse CRC organoids into fibroblast-specific knockout mice uncovered a novel SOX2-SRFP1 axis in CAFs that is crucial for TME reprogramming to promote tumorigenesis (Kasashima et al., 2021b). In this study, mice harboring

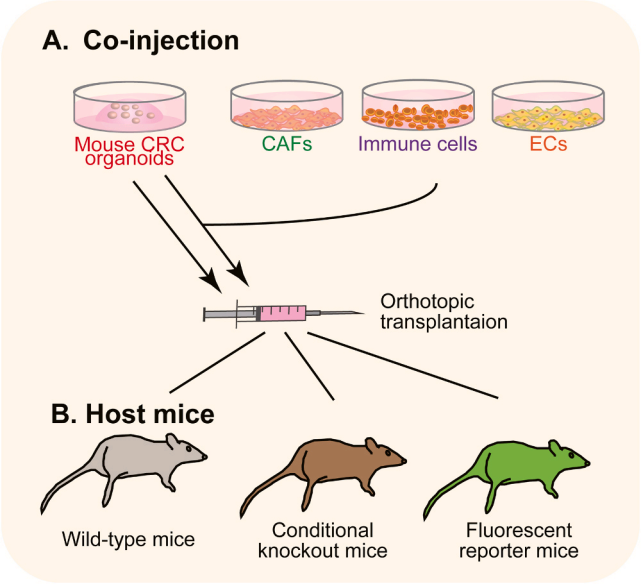


Fig. 3. Various options for orthotopic transplantation using co-injected grafts and transplanted hosts. Schematic of co-injection models using stromal cells (A) and transplanted host mice (B). (A) mouse CRC organoids can be co-injected with various stromal cells including CAFs, immune cells, and endothelial cells. Notably, these co-injected stromal cells as well as mouse CRC organoids can be genetically engineered prior to injection. (B) A wide variety of immunocompetent mice can be used as transplantation hosts depending on the objectives of the experiments. These mice include wild-type mice, conditional knockout mice, and fluorescent reporter mice.

fibroblast-specific conditional knockout of PKCζ were used as hosts for syngeneic transplantation into the cecum or the rectum. PKCζ is a member of the atypical protein kinase C (aPKC) family, which is known to regulate a variety of cancer types in a context-dependent manner (Reina-Campos et al., 2019). The loss of PKCζ in colon fibroblasts led to enlarged engrafted tumor sizes and increased metastasis incidence.

Notably, similar tumor-promoting effects were observed when mouse CRC organoids were co-implanted into wild-type mice with fibroblasts derived from *Prkcz*-knockout mice (Kasashima et al., 2021b). Furthermore, this tumor-promotive phenotype was canceled when either SOX2 or SFRP1 was deleted in PKCζ-deficient fibroblasts co-implanted with mouse CRC organoids. These findings not only reveal the pivotal role of PKCζ in the TME of CRCs, but also highlight the potential of co-implantation of tumor organoids with other stromal cells, including CAFs, ECs, and various types of immune cells (Fig. 3) (Kasashima et al., 2021a). These cells can be genetically engineered *in vitro*, independently of tumor organoids, to analyze crosstalk within the TME. Importantly, co-implantation is only applicable to mouse organoids, but not to human organoids, to preserve tumor immunity.

Similarly, serial orthotopic transplantation of mouse CRC organoids into genetically modified mice has contributed to another discovery of a novel myeloid-derived suppressor cell (MDSC) recruitment mechanism mediated by the THBS1-CXCL12-CXCR4 axis, which is responsible for suppressing $CD8^+$ T cell function (Omatsu et al., 2023). THBS1 is a matricellular protein typically expressed during inflammation or angiogenesis; however its role in tumorigenesis remains to be fully elucidated (Sid et al., 2004). Knockout of THBS1 in the host mouse showed suppressed metastatic capacity of orthotopically transplanted mouse CRC organoids as well as enhanced vascularity and $CD8^+$ T cell infiltration in the TME. Notably, CD36- or CD47-knockout mice mimicked the THBS1-meditated tumor suppressive effect, which was canceled by $CD8^+$ T cell depletion, suggesting that THBS1 suppresses T cell activity by binding to CD47 and CD36 on $CD8^+$ T cells. Orthotopic transplantation into myeloid-cell-specific THBS1-knockout mice replicated the tumor-suppressive effect, which is in good agreement with the scRNA-seq analyses, suggesting that monocytic MDSCs (MO-MDSCs) are the source of THBS1. Furthermore, chimeric mice expressing GFP specifically in bone marrow cells clearly demonstrated that THBS1-expressing myeloid cells in the TME were recruited from the bone marrow. Finally, THBS1 loss sensitized the transplanted tumors to ICI, indicating the potential of THBS1 as a biomarker for ICI response. As shown in this study, using fluorescent reporter mice as a transplantation host can be a strong tool to visualize the spatial distribution and the dynamics of cells in the TME, whereas knockout mice play an indispensable role in the functional analyses of genes and pathways (Fig. 3).

Syngeneic transplantation into myeloid-cell-specific knockout mice has unveiled another novel interaction between microbiota, polymorphonuclear MDSCs (PMN-MDSCs), and $CD8^+$ T cells mediated by the calcineurin-NFAT-IL-6 axis (Peuker et al., 2022). Orthotopic injection of mouse CRC organoids into mice with myeloid-cell-specific calcineurin knockout resulted in reduced tumor growth, increased death, and activated protective $CD8^+$ T cell responses. Inhibition of NFAT mimicked the tumor-suppressive phenotype, suggesting that NFAT mediates the tumor-promoting effects of myeloid calcineurin. Further antibody blockade experiments showed that myeloid calcineurin upregulates NFAT-mediated IL-6 expression, thereby promoting the expression of epithelial B7H3 and B7H4, which are co-inhibitory molecules associated with immune surveillance evasion (Lee et al., 2017; Li et al., 2018). Depletion of Ly6g positive myeloid cells caused tumor suppressive phenotypes in calcineurin-proficient mice but not in knockout mice, suggesting that PMN-MDSCs are the source of myeloid calcineurin and its tumor-promoting effects. This study has uncovered a new T cell response mechanism in MMR-proficient CRCs adding another layer of complexity to the multifaceted features of MDSCs (Veglia et al., 2021).

6. Future perspectives

Mouse CRC organoids, in collaboration with syngeneic transplantation models, have provided critical insights into CRC carcinogenesis, especially in the field of tumor immunity. However, challenges and issues remain to be addressed in certain subsets of CRCs. Thus, establishing mouse CRC organoid models is difficult when the original disease subsets are yet to be fully defined. For example, the serrated pathway is an alternative route for CRC development, typically originating from sessile serrated lesions (SSLs) as precancerous lesions, which account for approximately 30 % of overall CRCs (Szylberg et al., 2015). Whereas SSLs often have relatively indolent clinical course, dysplasia formation within the lesion (SSLDs) causes phenotypic changes that allow tumors to rapidly grow and develop into invasive cancers (Utsumi et al., 2023). While several groups have reported mouse models of CRCs with serrated features and their aggressive phenotypes, the heterogeneous nature of the disease has hampered efforts to create mouse SSL/SSLD organoids that can faithfully recapitulate the human serrated pathway (Bennecke et al., 2010; Carragher et al., 2010; DeStefano Shields et al., 2021; Jackstadt et al., 2019; Martinez-Ordoñez et al., 2023; Muta et al., 2023; Nakanishi et al., 2018; Sakamoto et al., 2017; Tong et al., 2021). Recent studies have discovered that metaplastic or fetal gene expression patterns are upregulated in the early stages of serrated tumor development, suggesting distinct cells of origin for serrated CRCs from conventional CRCs, following the adenoma-carcinoma sequence (Ayyaz et al., 2019; Chen et al., 2021; Joanito et al., 2022; Kinoshita et al., 2024; Martinez-Ordoñez et al., 2023). Given that PDOs derived from SSLs have already been established (Fujii et al., 2016), additional niche factors might be necessary for mouse SSL organoids. In fact, growing evidence supports that inflammation and the microbiota play pivotal roles in the emergence of serrated tumors, which might explain the discrepancy between human and mouse serrated tumors (Aiderus et al., 2024).

Likewise, it remains challenging for preclinical mouse models to fully reflect clinical factors such as tumor sidedness or companion diagnostics. The sidedness of primary tumors is of great clinical significance, because proximal (right-sided) CRCs are less sensitive to EGFR inhibition therapy and show worse overall survival compared to distal (left-sided) CRCs (Arnold et al., 2017; Petrelli et al., 2017). Some mouse tumor models have demonstrated predilections for the proximal colon; however, mouse proximal CRC organoids that are orthotopically transplantable remain to be developed (Leach et al., 2021; Martinez-Ordoñez et al., 2023; Muta et al., 2023; Nakanishi et al., 2018). Companion diagnostics for KRAS, BRAF, and HER2 are essential tools to predict the response to molecular-targeted therapy in unresectable CRCs (Cervantes et al., 2023). As HER2 amplification was initially identified as a potential therapeutic target by a large-scale PDX cohort (Bertotti et al., 2011), "top-down" approaches with PDOs from diverse patient backgrounds and scalable cohorts will be more useful in the discovery of novel therapeutic vulnerabilities. In contrast, mouse CRC organoids will make a greater contribution to deciphering the precise molecular mechanisms underlying clinical and epidemiological evidence.

Finally, interspecific differences in immunity might partially limit the validity of mouse CRC transplantation models. Humans and mice share many similarities in their genes, cell types, and pathophysiological activities, making mice the gold standard among mammalian model organisms. Nevertheless, some genes and immune cell subsets are only found in humans (Medetgul-Ernar and Davis, 2022). Considering the vast diversity and fine-tuned regulatory mechanisms of the immune system, these subtle differences might shape the distinct immune landscapes between mouse CRC organoids and corresponding human CRCs.

7. Conclusions

Mouse CRC organoids hold a unique position distinguished from human engineered CRC organoids or PDOs filling the gap between *in vitro* and *in vivo* system. Together with syngeneic orthotopic transplantation models, this system broadens our understanding of the complex landscape of the TME and provides fundamental insights into the development of novel therapeutic strategies for CRCs.

CRediT authorship contribution statement

Nakanishi Yuki: Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Muta Yu:** Writing – original draft, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Acknowledgements

Research was supported by the Japan Agency for Medical Research and Development (AMED), P-PROMOTE (22cm0106283h0002 (Y.N.), 23ama221326h0001 (Y.N.), 24ama221326h0002 (Y.N.)), the Fusion Oriented Research for disruptive Science and Technology (FOREST, 23719768 (Y.N.)), COI-NEXT (JPMJPF2018(Y.M.), the JSPS KAKENHI (JP24K18971 (Y.M.) and JP21H02902 (Y.N.)), the Foundations of Takeda Science (Y.M. and Y.N.), Mochida Memorial (Y.M.), Princess Takamatsu Cancer Research (Y.N.), Astellas, Daiichi Sankyo of Life Science (Y.N.).

References

Aiderus, A., Barker, N., Tergaonkar, V., 2024. Serrated colorectal cancer: preclinical models and molecular pathways. Trends Cancer 10, 76–91.

André, T., Shiu, K.K., Kim, T.W., Jensen, B.V., Jensen, L.H., Punt, C., Smith, D., Garcia-Carbonero, R., Benavides, M., Gibbs, P., de la Fouchardiere, C., Rivera, F., Elez, E., Bendell, J., Le, D.T., Yoshino, T., Van Cutsem, E., Yang, P., Farooqui, M.Z.H., Marinello, P., Diaz Jr., L.A., 2020. Pembrolizumab in microsatellite-instability-high advanced colorectal cancer. N. Engl. J. Med 383, 2207–2218.

Arnold, D., Lueza, B., Douillard, J.Y., Peeters, M., Lenz, H.J., Venook, A., Heinemann, V., Van Cutsem, E., Pignon, J.P., Tabernero, J., Cervantes, A., Ciardiello, F., 2017. Prognostic and predictive value of primary tumour side in patients with RAS wild-type metastatic colorectal cancer treated with chemotherapy and EGFR directed antibodies in six randomized trials. Ann. Oncol. 28, 1713–1729.

Ayyaz, A., Kumar, S., Sangiorgi, B., Ghoshal, B., Gosio, J., Ouladan, S., Fink, M., Barutcu, S., Trcka, D., Shen, J., Chan, K., Wrana, J.L., Gregorieff, A., 2019. Single-cell transcriptomes of the regenerating intestine reveal a revival stem cell. Nature 569, 121–125.

Bareham, B., Georgakopoulos, N., Matas-Céspedes, A., Curran, M., Saeb-Parsy, K., 2021. Modeling human tumor-immune environments in vivo for the preclinical assessment of immunotherapies. Cancer Immunol., Immunother. 70, 2737–2750.

Barker, N., van Es, J.H., Kuipers, J., Kujala, P., van den Born, M., Cozijnsen, M., Haegebarth, A., Korving, J., Begthel, H., Peters, P.J., Clevers, H., 2007. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 449, 1003–1007.

Bennecke, M., Kriegl, L., Bajbouj, M., Retzlaff, K., Robine, S., Jung, A., Arkan, M.C., Kirchner, T., Greten, F.R., 2010. Ink4a/Arf and oncogene-induced senescence prevent tumor progression during alternative colorectal tumorigenesis. Cancer Cell 18, 135–146.

Bertotti, A., Migliardi, G., Galimi, F., Sassi, F., Torti, D., Isella, C., Corà, D., Di Nicolantonio, F., Buscarino, M., Petti, C., Ribero, D., Russolillo, N., Muratore, A., Massucco, P., Pisacane, A., Molinaro, L., Valtorta, E., Sartore-Bianchi, A., Risio, M., Capussotti, L., Gambacorta, M., Siena, S., Medico, E., Sapino, A., Marsoni, S., Comoglio, P.M., Bardelli, A., Trusolino, L., 2011. A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discov. 1, 508–523.

Boonekamp, K.E., Kretzschmar, K., Wiener, D.J., Asra, P., Derakhshan, S., Puschhof, J., López-Iglesias, C., Peters, P.J., Basak, O., Clevers, H., 2019. Long-term expansion and differentiation of adult murine epidermal stem cells in 3D organoid cultures. Proc. Natl. Acad. Sci. 116, 14630–14638.

Branda, C.S., Dymecki, S.M., 2004. Talking about a revolution: the impact of site-specific recombinases on genetic analyses in mice. Dev. Cell 6, 7–28.

Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R.L., Soerjomataram, I., Jemal, A., 2024. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer J. Clin. 74, 229–263.

Cañellas-Socias, A., Cortina, C., Hernando-Momblona, X., Palomo-Ponce, S., Mulholland, E.J., Turon, G., Mateo, L., Conti, S., Roman, O., Sevillano, M., Slebe, F., Stork, D., Caballé-Mestres, A., Berenguer-Llergo, A., Álvarez-Varela, A., Fenderico, N., Novellasdemunt, L., Jiménez-Gracia, L., Sipka, T., Bardia, L., Lorden, P., Colombelli, J., Heyn, H., Trepat, X., Tejpar, S., Sancho, E., Tauriello, D.V. F., Leedham, S., Attolini, C.S., Batlle, E., 2022. Metastatic recurrence in colorectal cancer arises from residual EMP1(+) cells. Nature 611, 603–613.

Carragher, L.A., Snell, K.R., Giblett, S.M., Aldridge, V.S., Patel, B., Cook, S.J., Winton, D. J., Marais, R., Pritchard, C.A., 2010. V600EBraf induces gastrointestinal crypt senescence and promotes tumour progression through enhanced CpG methylation of p16INK4a. EMBO Mol. Med 2, 458–471.

Cercek, A., Lumish, M., Sinopoli, J., Weiss, J., Shia, J., Lamendola-Essel, M., El Dika, I.H., Segal, N., Shcherba, M., Sugarman, R., Stadler, Z., Yaeger, R., Smith, J.J., Rousseau, B., Argiles, G., Patel, M., Desai, A., Saltz, L.B., Widmar, M., Iyer, K., Zhang, J., Gianino, N., Crane, C., Romesser, P.B., Pappou, E.P., Paty, P., Garcia-Aguilar, J., Gonen, M., Gollub, M., Weiser, M.R., Schalper, K.A., Diaz Jr., L.A., 2022. PD-1 BLockade in Mismatch Repair-deficient, Locally Advanced Rectal Cancer. N. Engl. J. Med. 386, 2363–2376.

Cervantes, A., Adam, R., Roselló, S., Arnold, D., Normanno, N., Taïeb, J., Seligmann, J., De Baere, T., Osterlund, P., Yoshino, T., Martinelli, E., 2023. Metastatic colorectal cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up & #x2606. Ann. Oncol. 34, 10–32.

Chalabi, M., Fanchi, L.F., Dijkstra, K.K., Van den Berg, J.G., Aalbers, A.G., Sikorska, K., Lopez-Yurda, M., Grootscholten, C., Beets, G.L., Snaebjornsson, P., Maas, M., Mertz, M., Veninga, V., Bounova, G., Broeks, A., Beets-Tan, R.G., de Wijkerslooth, T. R., van Lent, A.U., Marsman, H.A., Nuijten, E., Kok, N.F., Kuiper, M., Verbeek, W.H., Kok, M., Van Leerdam, M.E., Schumacher, T.N., Voest, E.E., Haanen, J.B., 2020. Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. Nat. Med. 26, 566–576.

Chen, G., Huang, A.C., Zhang, W., Zhang, G., Wu, M., Xu, W., Yu, Z., Yang, J., Wang, B., Sun, H., Xia, H., Man, Q., Zhong, W., Antelo, L.F., Wu, B., Xiong, X., Liu, X., Guan, L., Li, T., Liu, S., Yang, R., Lu, Y., Dong, L., McGettigan, S., Somasundaram, R., Radhakrishnan, R., Mills, G., Lu, Y., Kim, J., Chen, Y.H., Dong, H., Zhao, Y., Karakousis, G.C., Mitchell, T.C., Schuchter, L.M., Herlyn, M., Wherry, E.J., Xu, X., Guo, W., 2018. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. Nature 560, 382–386.

Chen, B., Scurrah, C.R., McKinley, E.T., Simmons, A.J., Ramirez-Solano, M.A., Zhu, X., Markham, N.O., Heiser, C.N., Vega, P.N., Rolong, A., Kim, H., Sheng, Q., Drewes, J. L., Zhou, Y., Southard-Smith, A.N., Xu, Y., Ro, J., Jones, A.L., Revetta, F., Berry, L.D., Niitsu, H., Islam, M., Pelka, K., Hofree, M., Chen, J.H., Sarkizova, S., Ng, K., Giannakis, M., Boland, G.M., Aguirre, A.J., Anderson, A.C., Rozenblatt-Rosen, O., Regev, A., Hacohen, N., Kawasaki, K., Sato, T., Goettel, J.A., Grady, W.M., Zheng, W., Washington, M.K., Cai, Q., Sears, C.L., Goldenring, J.R., Franklin, J.L., Su, T., Huh, W.J., Vandekar, S., Roland, J.T., Liu, Q., Coffey, R.J., Shrubsole, M.J., Lau, K.S., 2021. Differential pre-malignant programs and microenvironment chart distinct paths to malignancy in human colorectal polyps. Cell 184, 6262–6280 e6226.

Cheung, P., Xiol, J., Dill, M.T., Yuan, W.-C., Panero, R., Roper, J., Osorio, F.G., Maglic, D., Li, Q., Gurung, B., Calogero, R.A., Yilmaz, Ö.H., Mao, J., Camargo, F.D., 2020. Regenerative reprogramming of the intestinal stem cell state via hippo signaling suppresses metastatic colorectal cancer. Cell Stem Cell 27, 590–604 e599.

Chuprin, J., Buettner, H., Seedhom, M.O., Greiner, D.L., Keck, J.G., Ishikawa, F., Shultz, L.D., Brehm, M.A., 2023. Humanized mouse models for immuno-oncology research. Nat. Rev. Clin. Oncol. 20, 192–206.

Ciardiello, F., Ciardiello, D., Martini, G., Napolitano, S., Tabernero, J., Cervantes, A., 2022. Clinical management of metastatic colorectal cancer in the era of precision medicine. CA Cancer J. Clin. 72, 372–401.

Corbett, T.H., Griswold Jr., D.P., Roberts, B.J., Peckham, J.C., Schabel Jr., F.M., 1975. Tumor induction relationships in development of transplantable cancers of the colon in mice for chemotherapy assays, with a note on carcinogen structure. Cancer Res 35, 2434–2439.

Dekker, E., Tanis, P.J., Vleugels, J.L.A., Kasi, P.M., Wallace, M.B., 2019. Colorectal cancer. Lancet 394, 1467–1480.

van der Flier, L.G., Clevers, H., 2009. Stem cells, self-renewal, and differentiation in the intestinal epithelium. Annu. Rev. Physiol. 71, 241–260.

DeStefano Shields, C.E., White, J.R., Chung, L., Wenzel, A., Hicks, J.L., Tam, A.J., Chan, J.L., Dejea, C.M., Fan, H., Michel, J., Maiuri, A.R., Sriramkumar, S., Podicheti, R., Rusch, D.B., Wang, H., De Marzo, A.M., Besharati, S., Anders, R.A., Baylin, S.B., O'Hagan, H.M., Housseau, F., Sears, C.L., 2021. Bacterial-driven inflammation and mutant BRAF expression combine to promote murine colon tumorigenesis that is sensitive to immune checkpoint therapy. Cancer Discov. 11, 1792–1807.

Dijkstra, K.K., Cattaneo, C.M., Weeber, F., Chalabi, M., van de Haar, J., Fanchi, L.F., Slagter, M., van der Velden, D.L., Kaing, S., Kelderman, S., van Rooij, N., van Leerdam, M.E., Depla, A., Smit, E.F., Hartemink, K.J., de Groot, R., Wolkers, M.C., Sachs, N., Snaebjornsson, P., Monkhorst, K., Haanen, J., Clevers, H., Schumacher, T. N., Voest, E.E., 2018. Generation of tumor-reactive T cells by Co-culture of peripheral blood lymphocytes and tumor organoids. Cell 174, 1586–1598 e1512.

Ding, S., Hsu, C., Wang, Z., Natesh, N.R., Millen, R., Negrete, M., Giroux, N., Rivera, G. O., Dohlman, A., Bose, S., Rotstein, T., Spiller, K., Yeung, A., Sun, Z., Jiang, C., Xi, R., Wilkin, B., Randon, P.M., Williamson, I., Nelson, D.A., Delubac, D., Oh, S., Rupprecht, G., Isaacs, J., Jia, J., Chen, C., Shen, J.P., Kopetz, S., McCall, S., Smith, A., Gjorevski, N., Walz, A.C., Antonia, S., Marrer-Berger, E., Clevers, H., Hsu, D., Shen, X., 2022. Patient-derived micro-organospheres enable clinical precision oncology. Cell Stem Cell 29, 905–917 e906.

Dow, Lukas E., O'Rourke, Kevin P., Simon, J., Tschaharganeh, Darjus F., van Es, Johan H., Clevers, H., Lowe, Scott W., 2015. Apc restoration promotes cellular differentiation and reestablishes crypt homeostasis in colorectal cancer. Cell 161, 1539–1552.

Drost, J., van Jaarsveld, R.H., Ponsioen, B., Zimberlin, C., van Boxtel, R., Buijs, A., Sachs, N., Overmeer, R.M., Offerhaus, G.J., Begthel, H., Korving, J., van de Wetering, M., Schwank, G., Logtenberg, M., Cuppen, E., Snippert, H.J., Medema, J.

P., Kops, G.J.P.L., Clevers, H., 2015. Sequential cancer mutations in cultured human intestinal stem cells. Nature 521, 43–47.
Felchle, H., Brunner, V., Groll, T., Walther, C.N., Nefzger, S.M., Zaurito, A.E., Silva, M.G., Gissibl, J., Topping, G.J., Lansink Rotgerink, L., Saur, D., Steiger, K., Combs, S.E., Tschurtschenthaler, M., Fischer, J.C., 2024. Novel tumor organoid-based mouse model to study image guided radiation therapy of rectal cancer after noninvasive and precise endoscopic implantation. Int. J. Radiat. Oncol., Biol., Phys. 118, 1094–1104.
Fu, X., Besterman, J.M., Monosov, A., Hoffman, R.M., 1991. Models of human metastatic colon cancer in nude mice orthotopically constructed by using histologically intact patient specimens. Proc. Natl. Acad. Sci. 88, 9345–9349.
Fujii, M., Matano, M., Toshimitsu, K., Takano, A., Mikami, Y., Nishikori, S., Sugimoto, S., Sato, T., 2018. Human intestinal organoids maintain self-renewal capacity and cellular diversity in niche-inspired culture condition. Cell Stem Cell 23, 787–793 e786.
Fujii, M., Sato, T., 2021. Somatic cell-derived organoids as prototypes of human epithelial tissues and diseases. Nat. Mater. 20, 156–169.
Fujii, M., Shimokawa, M., Date, S., Takano, A., Matano, M., Nanki, K., Ohta, Y., Toshimitsu, K., Nakazato, Y., Kawasaki, K., Uraoka, T., Watanabe, T., Kanai, T., Sato, T., 2016. A Colorectal tumor organoid library demonstrates progressive loss of niche factor requirements during tumorigenesis. Cell Stem Cell 18, 827–838.
Fumagalli, A., Drost, J., Suijkerbuijk, S.J., van Boxtel, R., de Ligt, J., Offerhaus, G.J., Begthel, H., Beerling, E., Tan, E.H., Sansom, O.J., Cuppen, E., Clevers, H., van Rheenen, J., 2017. Genetic dissection of colorectal cancer progression by orthotopic transplantation of engineered cancer organoids. Proc. Natl. Acad. Sci. USA 114, E2357–e2364.
Fumagalli, A., Oost, K.C., Kester, L., Morgner, J., Bornes, L., Bruens, L., Spaargaren, L., Azkanaz, M., Schelfhorst, T., Beerling, E., Heinz, M.C., Postrach, D., Seinstra, D., Sieuwerts, A.M., Martens, J.W.M., van der Elst, S., van Baalen, M., Bhowmick, D., Vrisekoop, N., Ellenbroek, S.I.J., Suijkerbuijk, S.J.E., Snippert, H.J., van Rheenen, J., 2020. Plasticity of Lgr5-negative cancer cells drives metastasis in colorectal cancer. Cell Stem Cell 26, 569–578 e567.
Fumagalli, A., Suijkerbuijk, S.J.E., Begthel, H., Beerling, E., Oost, K.C., Snippert, H.J., van Rheenen, J., Drost, J., 2018. A surgical orthotopic organoid transplantation approach in mice to visualize and study colorectal cancer progression. Nat. Protoc. 13, 235–247.
Ganesh, K., Basnet, H., Kaygusuz, Y., Laughney, A.M., He, L., Sharma, R., O'Rourke, K.P., Reuter, V.P., Huang, Y.-H., Turkekul, M., Er, E.E., Masilionis, I., Manova-Todorova, K., Weiser, M.R., Saltz, L.B., Garcia-Aguilar, J., Koche, R., Lowe, S.W., Pe'er, D., Shia, J., Massagué, J., 2020. L1CAM defines the regenerative origin of metastasis-initiating cells in colorectal cancer. Nat. Cancer 1, 28–45.
Ganesh, K., Wu, C., O'Rourke, K.P., Szeglin, B.C., Zheng, Y., Sauvé, C.G., Adileh, M., Wasserman, I., Marco, M.R., Kim, A.S., Shady, M., Sanchez-Vega, F., Karthaus, W.R., Won, H.H., Choi, S.H., Pelossof, R., Barlas, A., Ntiamoah, P., Pappou, E., Elghouayel, A., Strong, J.S., Chen, C.T., Harris, J.W., Weiser, M.R., Nash, G.M., Guillem, J.G., Wei, I.H., Kolesnick, R.N., Veeraraghavan, H., Ortiz, E.J., Petkovska, I., Cercek, A., Manova-Todorova, K.O., Saltz, L.B., Lavery, J.A., DeMatteo, R.P., Massagué, J., Paty, P.B., Yaeger, R., Chen, X., Patil, S., Clevers, H., Berger, M.F., Lowe, S.W., Shia, J., Romesser, P.B., Dow, L.E., Garcia-Aguilar, J., Sawyers, C.L., Smith, J.J., 2019. A rectal cancer organoid platform to study individual responses to chemoradiation. Nat. Med 25, 1607–1614.
Gerarduzzi, C., Hartmann, U., Leask, A., Drobetsky, E., 2020. The matrix revolution: matricellular proteins and restructuring of the cancer microenvironment. Cancer Res. 80, 2705–2717.
Geurts, M.H., Clevers, H., 2023. CRISPR engineering in organoids for gene repair and disease modelling. Nat. Rev. Bioeng. 1, 32–45.
Goto, N., Westcott, P.M.K., Goto, S., Imada, S., Taylor, M.S., Eng, G., Braverman, J., Deshpande, V., Jacks, T., Agudo, J., Yilmaz, Ö.H., 2024. SOX17 enables immune evasion of early colorectal adenomas and cancers. Nature 627, 636–645.
Herpers, B., Eppink, B., James, M.I., Cortina, C., Cañellas-Socias, A., Boj, S.F., Hernando-Momblona, X., Glodzik, D., Roovers, R.C., van de Wetering, M., Bartelink-Clements, C., Zondag-van der Zande, V., Mateos, J.G., Yan, K., Salinaro, L., Basmeleh, A., Fatrai, S., Maussang, D., Lammerts van Bueren, J.J., Chicote, I., Serna, G., Cabellos, L., Ramírez, L., Nuciforo, P., Salazar, R., Santos, C., Villanueva, A., Stephan-Otto Attolini, C., Sancho, E., Palmer, H.G., Tabernero, J., Stratton, M.R., de Kruif, J., Logtenberg, T., Clevers, H., Price, L.S., Vries, R.G.J., Batlle, E., Throsby, M., 2022. Functional patient-derived organoid screenings identify MCLA-158 as a therapeutic EGFR × LGR5 bispecific antibody with efficacy in epithelial tumors. Nat. Cancer 3, 418–436.
Hesselton, R.M., Greiner, D.L., Mordes, J.P., Rajan, T.V., Sullivan, J.L., Shultz, L.D., 1995. High levels of human peripheral blood mononuclear cell engraftment and enhanced susceptibility to human immunodeficiency virus type 1 infection in NOD/LtSz-scid/scid mice. J. Infect. Dis. 172, 974–982.
Huch, M., Bonfanti, P., Boj, S.F., Sato, T., Loomans, C.J.M., van de Wetering, M., Sojoodi, M., Li, V.S.W., Schuijers, J., Gracanin, A., Ringnalda, F., Begthel, H., Hamer, K., Mulder, J., van Es, J.H., de Koning, E., Vries, R.G.J., Heimberg, H., Clevers, H., 2013a. Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. EMBO J. 32, 2708–2721.
Huch, M., Dorrell, C., Boj, S.F., Van Es, J.H., Li, V.S., Van De Wetering, M., Sato, T., Hamer, K., Sasaki, N., Finegold, M.J., 2013b. In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. Nature 494, 247–250.
Ito, M., Hiramatsu, H., Kobayashi, K., Suzue, K., Kawahata, M., Hioki, K., Ueyama, Y., Koyanagi, Y., Sugamura, K., Tsuji, K., Heike, T., Nakahata, T., 2002. NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. Blood 100, 3175–3182.
Jackstadt, R., van Hooff, S.R., Leach, J.D., Cortes-Lavaud, X., Lohuis, J.O., Ridgway, R.A., Wouters, V.M., Roper, J., Kendall, T.J., Roxburgh, C.S., Horgan, P.G., Nixon, C., Nourse, C., Gunzer, M., Clark, W., Hedley, A., Yilmaz, O.H., Rashid, M., Bailey, P., Biankin, A.V., Campbell, A.D., Adams, D.J., Barry, S.T., Steele, C.W., Medema, J.P., Sansom, O.J., 2019. Epithelial NOTCH signaling rewires the tumor microenvironment of colorectal cancer to drive poor-prognosis subtypes and metastasis. Cancer Cell 36, 319–336 e317.
Jenkins, M.A., Hayashi, S., O'Shea, A.-M., Burgart, L.J., Smyrk, T.C., Shimizu, D., Waring, P.M., Ruszkiewicz, A.R., Pollett, A.F., Redston, M., Barker, M.A., Baron, J. A., Casey, G.R., Dowty, J.G., Giles, G.G., Limburg, P., Newcomb, P., Young, J.P., Walsh, M.D., Thibodeau, S.N., Lindor, N.M., LeMarchand, L., Gallinger, S., Haile, R. W., Potter, J.D., Hopper, J.L., Jass, J.R., 2007. Pathology features in Bethesda guidelines predict colorectal cancer microsatellite instability: a population-based study. Gastroenterology 133, 48–56.
Joanito, I., Wirapati, P., Zhao, N., Nawaz, Z., Yeo, G., Lee, F., Eng, C.L.P., Macalinao, D. C., Kahraman, M., Srinivasan, H., Lakshmanan, V., Verbandt, S., Tsantoulis, P., Gunn, N., Venkatesh, P.N., Poh, Z.W., Nahar, R., Oh, H.L.J., Loo, J.M., Chia, S., Cheow, L.F., Cheruba, E., Wong, M.T., Kua, L., Chua, C., Nguyen, A., Golovan, J., Gan, A., Lim, W.J., Guo, Y.A., Yap, C.K., Tay, B., Hong, Y., Chong, D.Q., Chok, A.Y., Park, W.Y., Han, S., Chang, M.H., Seow-En, I., Fu, C., Mathew, R., Toh, E.L., Hong, L. Z., Skanderup, A.J., DasGupta, R., Ong, C.J., Lim, K.H., Tan, E.K.W., Koo, S.L., Leow, W.Q., Tejpar, S., Prabhakar, S., Tan, I.B., 2022. Single-cell and bulk transcriptome sequencing identifies two epithelial tumor cell states and refines the consensus molecular classification of colorectal cancer. Nat. Genet 54, 963–975.
Kasashima, H., Duran, A., Cid-Diaz, T., Muta, Y., Kinoshita, H., Batlle, E., Diaz-Meco, M. T., Moscat, J., 2021a. Mouse model of colorectal cancer: orthotopic co-implantation of tumor and stroma cells in cecum and rectum. STAR Protoc. 2, 100297.
Kasashima, H., Duran, A., Martinez-Ordoñez, A., Nakanishi, Y., Kinoshita, H., Linares, J. F., Reina-Campos, M., Kudo, Y., L'Hermitte, A., Yashiro, M., Ohira, M., Bao, F., Tauriello, D.V.F., Batlle, E., Diaz-Meco, M.T., Moscat, J., 2021b. Stromal SOX2 upregulation promotes tumorigenesis through the generation of a SFRP1/2-expressing cancer-associated fibroblast population. Dev. Cell 56, 95–110 e110.
Kinoshita, H., Martinez-Ordoñez, A., Cid-Diaz, T., Han, Q., Duran, A., Muta, Y., Zhang, X., Linares, J.F., Nakanishi, Y., Kasashima, H., Yashiro, M., Maeda, K., Albaladejo-Gonzalez, A., Torres-Moreno, D., García-Solano, J., Conesa-Zamora, P., Inghirami, G., Diaz-Meco, M.T., Moscat, J., 2024. Epithelial aPKC deficiency leads to stem cell loss preceding metaplasia in colorectal cancer initiation. Dev. Cell 59, 1972–1987 e1978.
Kong, J., Lee, H., Kim, D., Han, S.K., Ha, D., Shin, K., Kim, S., 2020. Network-based machine learning in colorectal and bladder organoid models predicts anti-cancer drug efficacy in patients. Nat. Commun. 11, 5485.
Kucherlapati, M.H., 2023. Mouse models in colon cancer, inferences, and implications. iScience 26, 106958.
Le, D.T., Durham, J.N., Smith, K.N., Wang, H., Bartlett, B.R., Aulakh, L.K., Lu, S., Kemberling, H., Wilt, C., Luber, B.S., Wong, F., Azad, N.S., Rucki, A.A., Laheru, D., Donehower, R., Zaheer, A., Fisher, G.A., Crocenzi, T.S., Lee, J.J., Greten, T.F., Duffy, A.G., Ciombor, K.K., Eyring, A.D., Lam, B.H., Joe, A., Kang, S.P., Holdhoff, M., Danilova, L., Cope, L., Meyer, C., Zhou, S., Goldberg, R.M., Armstrong, D.K., Bever, K.M., Fader, A.N., Taube, J., Housseau, F., Spetzler, D., Xiao, N., Pardoll, D. M., Papadopoulos, N., Kinzler, K.W., Eshleman, J.R., Vogelstein, B., Anders, R.A., Diaz Jr., L.A., 2017. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 357, 409–413.
Le, D.T., Uram, J.N., Wang, H., Bartlett, B.R., Kemberling, H., Eyring, A.D., Skora, A.D., Luber, B.S., Azad, N.S., Laheru, D., Biedrzycki, B., Donehower, R.C., Zaheer, A., Fisher, G.A., Crocenzi, T.S., Lee, J.J., Duffy, S.M., Goldberg, R.M., de la Chapelle, A., Koshiji, M., Bhaijee, F., Huebner, T., Hruban, R.H., Wood, L.D., Cuka, N., Pardoll, D. M., Papadopoulos, N., Kinzler, K.W., Zhou, S., Cornish, T.C., Taube, J.M., Anders, R. A., Eshleman, J.R., Vogelstein, B., Diaz Jr., L.A., 2015. PD-1 blockade in tumors with mismatch-repair deficiency. N. Engl. J. Med. 372, 2509–2520.
Leach, J.D.G., Vlahov, N., Tsantoulis, P., Ridgway, R.A., Flanagan, D.J., Gilroy, K., Sphyris, N., Vázquez, E.G., Vincent, D.F., Faller, W.J., Hodder, M.C., Raven, A., Fey, S., Najumudeen, A.K., Strathdee, D., Nixon, C., Hughes, M., Clark, W., Shaw, R., van Hooff, S.R., Huels, D.J., Medema, J.P., Barry, S.T., Frame, M.C., Unciti-Broceta, A., Leedham, S.J., Inman, G.J., Jackstadt, R., Thompson, B.J., Campbell, A. D., Tejpar, S., Sansom, O.J., 2021. Oncogenic BRAF, unrestrained by TGFβ-receptor signalling, drives right-sided colonic tumorigenesis. Nat. Commun. 12, 3464.
Lee, Y.-h, Martin-Orozco, N., Zheng, P., Li, J., Zhang, P., Tan, H., Park, H.J., Jeong, M., Chang, S.H., Kim, B.-S., Xiong, W., Zang, W., Guo, L., Liu, Y., Dong, Z.-j, Overwijk, W.W., Hwu, P., Yi, Q., Kwak, L., Yang, Z., Mak, T.W., Li, W., Radvanyi, L. G., Ni, L., Liu, D., Dong, C., 2017. Inhibition of the B7-H3 immune checkpoint limits tumor growth by enhancing cytotoxic lymphocyte function. Cell Res. 27, 1034–1045.
Lenz, H.J., Van Cutsem, E., Luisa Limon, M., Wong, K.Y.M., Hendlisz, A., Aglietta, M., García-Alfonso, P., Neyns, B., Luppi, G., Cardin, D.B., Dragovich, T., Shah, U., Abdullaev, S., Gricar, J., Ledeine, J.M., Overman, M.J., Lonardi, S., 2022. First-line nivolumab plus low-dose ipilimumab for microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: the phase II CheckMate 142 study. J. Clin. Oncol. 40, 161–170.
Li, J., Lee, Y., Li, Y., Jiang, Y., Lu, H., Zang, W., Zhao, X., Liu, L., Chen, Y., Tan, H., Yang, Z., Zhang, M.Q., Mak, T.W., Ni, L., Dong, C., 2018. Co-inhibitory molecule B7 superfamily member 1 expressed by tumor-infiltrating myeloid cells induces dysfunction of anti-tumor CD8(+) T cells. Immunity 48, 773–786 e775.
Li, N., Zhu, Q., Tian, Y., Ahn, K.J., Wang, X., Cramer, Z., Jou, J., Folkert, I.W., Yu, P., Adams-Tzivelekidis, S., Sehgal, P., Mahmoud, N.N., Aarons, C.B., Roses, R.E., Thomas-Tikhonenko, A., Furth, E.E., Stanger, B.Z., Rustgi, A., Haldar, M., Katona, B.

W., Tan, K., Lengner, C.J., 2023. Mapping and modeling human colorectal carcinoma interactions with the tumor microenvironment. Nat. Commun. 14, 7915.
Lo, Y.-H., Karlsson, K., Kuo, C.J., 2020. Applications of organoids for cancer biology and precision medicine. Nat. Cancer 1, 761–773.
Lucotti, S., Kenific, C.M., Zhang, H., Lyden, D., 2022a. Extracellular vesicles and particles impact the systemic landscape of cancer. Embo J. 41, e109288.
Lucotti, S., Kenific, C.M., Zhang, H., Lyden, D., 2022b. Extracellular vesicles and particles impact the systemic landscape of cancer. EMBO J. 41, e109288.
Makino, S., Kunimoto, K., Muraoka, Y., Mizushima, Y., Katagiri, K., Tochino, Y., 1980. Breeding of a non-obese, diabetic strain of mice. Exp. Anim. 29, 1–13.
Martinez-Ordoñez, A., Duran, A., Ruiz-Martinez, M., Cid-Diaz, T., Zhang, X., Han, Q., Kinoshita, H., Muta, Y., Linares, J.F., Kasashima, H., Nakanishi, Y., Omar, M., Nishimura, S., Avila, L., Yashiro, M., Maeda, K., Pannellini, T., Pigazzi, A., Inghirami, G., Marchionni, L., Sigal, D., Diaz-Meco, M.T., Moscat, J., 2023. Hyaluronan driven by epithelial aPKC deficiency remodels the microenvironment and creates a vulnerability in mesenchymal colorectal cancer. Cancer Cell 41, 252–271 e259.
Masters, J.R., 2000. Human cancer cell lines: fact and fantasy. Nat. Rev. Mol. Cell Biol. 1, 233–236.
Matano, M., Date, S., Shimokawa, M., Takano, A., Fujii, M., Ohta, Y., Watanabe, T., Kanai, T., Sato, T., 2015. Modeling colorectal cancer using CRISPR-Cas9–mediated engineering of human intestinal organoids. Nat. Med. 21, 256–262.
Mauri, G., Durinikova, E., Amatu, A., Tosi, F., Cassingena, A., Rizzetto, F., Buzo, K., Arcella, P., Aquilano, M.C., Bonoldi, E., Marsoni, S., Siena, S., Bardelli, A., Sartore-Bianchi, A., Arena, S., 2021. Empowering clinical decision making in oligometastatic colorectal cancer: the potential role of drug screening of patient-derived organoids. JCO Precis Oncol. 5.
Medetgul-Ernar, K., Davis, M.M., 2022. Standing on the shoulders of mice. Immunity 55, 1343–1353.
de Miguel, M., Calvo, E., 2020. Clinical challenges of immune checkpoint inhibitors. Cancer Cell 38, 326–333.
Moser, A.R., Pitot, H.C., Dove, W.F., 1990. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science 247, 322–324.
Muta, Y., Linares, J.F., Martinez-Ordoñez, A., Duran, A., Cid-Diaz, T., Kinoshita, H., Zhang, X., Han, Q., Nakanishi, Y., Nakanishi, N., Cordes, T., Arora, G.K., Ruiz-Martinez, M., Reina-Campos, M., Kasashima, H., Yashiro, M., Maeda, K., Albaladejo-Gonzalez, A., Torres-Moreno, D., García-Solano, J., Conesa-Zamora, P., Inghirami, G., Metallo, C.M., Osborne, T.F., Diaz-Meco, M.T., Moscat, J., 2023. Enhanced SREBP2-driven cholesterol biosynthesis by PKCλ/ι deficiency in intestinal epithelial cells promotes aggressive serrated tumorigenesis. Nat. Commun. 14, 8075.
Nakanishi, Y., Duran, A., L'Hermitte, A., Shelton, P.M., Nakanishi, N., Reina-Campos, M., Huang, J., Soldevila, F., Baaten, B.J.G., Tauriello, D.V.F., Castilla, E.A., Bhangoo, M. S., Bao, F., Sigal, D., Diaz-Meco, M.T., Moscat, J., 2018. Simultaneous loss of both atypical protein kinase C genes in the intestinal epithelium drives serrated intestinal cancer by impairing immunosurveillance. Immunity 49, 1132–1147 e1137.
Neal, J.T., Li, X., Zhu, J., Giangarra, V., Grzeskowiak, C.L., Ju, J., Liu, I.H., Chiou, S.-H., Salahudeen, A.A., Smith, A.R., Deutsch, B.C., Liao, L., Zemek, A.J., Zhao, F., Karlsson, K., Schultz, L.M., Metzner, T.J., Nadauld, L.D., Tseng, Y.-Y., Alkhairy, S., Oh, C., Keskula, P., Mendoza-Villanueva, D., De La Vega, F.M., Kunz, P.L., Liao, J.C., Leppert, J.T., Sunwoo, J.B., Sabatti, C., Boehm, J.S., Hahn, W.C., Zheng, G.X.Y., Davis, M.M., Kuo, C.J., 2018. Organoid modeling of the tumor immune microenvironment. Cell 175, 1972–1988 e1916.
Nors, J., Iversen, L.H., Erichsen, R., Gotschalck, K.A., Andersen, C.L., 2024. Incidence of recurrence and time to recurrence in stage I to III colorectal cancer: a Nationwide Danish Cohort Study. JAMA Oncol. 10, 54–62.
Omatsu, M., Nakanishi, Y., Iwane, K., Aoyama, N., Duran, A., Muta, Y., Martinez-Ordoñez, A., Han, Q., Agatsuma, N., Mizukoshi, K., Kawai, M., Yamakawa, G., Namikawa, M., Hamada, K., Fukunaga, Y., Utsumi, T., Sono, M., Masuda, T., Hata, A., Araki, O., Nagao, M., Yoshikawa, T., Ogawa, S., Hiramatsu, Y., Tsuda, M., Maruno, T., Kogame, T., Kasashima, H., Kakiuchi, N., Nakagawa, M.M., Kawada, K., Yashiro, M., Maeda, K., Saito, Y., Matozaki, T., Fukuda, A., Kabashima, K., Obama, K., Ogawa, S., Sheibani, N., Diaz-Meco, M.T., Moscat, J., Seno, H., 2023. THBS1-producing tumor-infiltrating monocyte-like cells contribute to immunosuppression and metastasis in colorectal cancer. Nat. Commun. 14, 5534.
Ooft, S.N., Weeber, F., Dijkstra, K.K., McLean, C.M., Kaing, S., van Werkhoven, E., Schipper, L., Hoes, L., Vis, D.J., van de Haar, J., Prevoo, W., Snaebjornsson, P., van der Velden, D., Klein, M., Chalabi, M., Boot, H., van Leerdam, M., Bloemendal, H.J., Beerepoot, L.V., Wessels, L., Cuppen, E., Clevers, H., Voest, E.E., 2019. Patient-derived organoids can predict response to chemotherapy in metastatic colorectal cancer patients. Sci. Transl. Med 11.
Ooft, S.N., Weeber, F., Schipper, L., Dijkstra, K.K., McLean, C.M., Kaing, S., van de Haar, J., Prevoo, W., van Werkhoven, E., Snaebjornsson, P., Hoes, L.R., Chalabi, M., van der Velden, D., van Leerdam, M., Boot, H., Grootscholten, C., Huitema, A.D.R., Bloemendal, H.J., Cuppen, E., Voest, E.E., 2021. Prospective experimental treatment of colorectal cancer patients based on organoid drug responses. ESMO Open 6, 100103.
Ootani, A., Li, X., Sangiorgi, E., Ho, Q.T., Ueno, H., Toda, S., Sugihara, H., Fujimoto, K., Weissman, I.L., Capecchi, M.R., Kuo, C.J., 2009. Sustained in vitro intestinal epithelial culture within a Wnt-dependent stem cell niche. Nat. Med 15, 701–706.
O'Rourke, K.P., Loizou, E., Livshits, G., Schatoff, E.M., Baslan, T., Manchado, E., Simon, J., Romesser, P.B., Leach, B., Han, T., Pauli, C., Beltran, H., Rubin, M.A., Dow, L.E., Lowe, S.W., 2017. Transplantation of engineered organoids enables rapid generation of metastatic mouse models of colorectal cancer. Nat. Biotechnol. 35, 577–582.
Overman, M.J., Lonardi, S., Wong, K.Y.M., Lenz, H.J., Gelsomino, F., Aglietta, M., Morse, M.A., Van Cutsem, E., McDermott, R., Hill, A., Sawyer, M.B., Hendlisz, A., Neyns, B., Svrcek, M., Moss, R.A., Ledeine, J.M., Cao, Z.A., Kamble, S., Kopetz, S., André, T., 2018. Durable clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair-deficient/microsatellite instability-high metastatic colorectal cancer. J. Clin. Oncol. 36, 773–779.
Park, M., Kwon, J., Kong, J., Moon, S.M., Cho, S., Yang, K.Y., Jang, W.I., Kim, M.S., Kim, Y., Shin, U.S., 2021. A patient-derived organoid-based radiosensitivity model for the prediction of radiation responses in patients with rectal cancer. Cancers 13.
Peinado, H., Alečković, M., Lavotshkin, S., Matei, I., Costa-Silva, B., Moreno-Bueno, G., Hergueta-Redondo, M., Williams, C., García-Santos, G., Ghajar, C., Nitadori-Hoshino, A., Hoffman, C., Badal, K., Garcia, B.A., Callahan, M.K., Yuan, J., Martins, V.R., Skog, J., Kaplan, R.N., Brady, M.S., Wolchok, J.D., Chapman, P.B., Kang, Y., Bromberg, J., Lyden, D., 2012. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. Nat. Med 18, 883–891.
Petrelli, F., Tomasello, G., Borgonovo, K., Ghidini, M., Turati, L., Dallera, P., Passalacqua, R., Sgroi, G., Barni, S., 2017. Prognostic survival associated with left-sided vs right-sided colon cancer: a systematic review and meta-analysis. JAMA Oncol. 3, 211–219.
Peuker, K., Strigli, A., Tauriello, D.V.F., Hendricks, A., von Schönfels, W., Burmeister, G., Brosch, M., Herrmann, A., Krüger, S., Nitsche, J., Južnić, L., Geissler, M.M., Hiergeist, A., Gessner, A., Wirbel, J., Ponnudurai, R.P., Tunger, A., Wehner, R., Stange, D.E., Weitz, J., Aust, D.E., Baretton, G.B., Schmitz, M., Röcken, C., Hampe, J., Hinz, S., Zeller, G., Chavakis, T., Schafmayer, C., Batlle, E., Zeissig, S., 2022. Microbiota-dependent activation of the myeloid calcineurin-NFAT pathway inhibits B7H3- and B7H4-dependent anti-tumor immunity in colorectal cancer. Immunity 55, 701–717 e707.
Poggio, M., Hu, T., Pai, C.C., Chu, B., Belair, C.D., Chang, A., Montabana, E., Lang, U.E., Fu, Q., Fong, L., Blelloch, R., 2019. Suppression of exosomal PD-L1 induces systemic anti-tumor immunity and memory. Cell 177, 414–427 e413.
Reina-Campos, M., Diaz-Meco, M.T., Moscat, J., 2019. The dual roles of the atypical protein kinase Cs in cancer. Cancer Cell 36, 218–235.
Roper, J., Tammela, T., Akkad, A., Almeqdadi, M., Santos, S.B., Jacks, T., Yilmaz, Ö.H., 2018. Colonoscopy-based colorectal cancer modeling in mice with CRISPR–Cas9 genome editing and organoid transplantation. Nat. Protoc. 13, 217–234.
Roper, J., Tammela, T., Cetinbas, N.M., Akkad, A., Roghanian, A., Rickelt, S., Almeqdadi, M., Wu, K., Oberli, M.A., Sánchez-Rivera, F.J., Park, Y.K., Liang, X., Eng, G., Taylor, M.S., Azimi, R., Kedrin, D., Neupane, R., Beyaz, S., Sicinska, E.T., Suarez, Y., Yoo, J., Chen, L., Zukerberg, L., Katajisto, P., Deshpande, V., Bass, A.J., Tsichlis, P.N., Lees, J., Langer, R., Hynes, R.O., Chen, J., Bhutkar, A., Jacks, T., Yilmaz, Ö.H., 2017. In vivo genome editing and organoid transplantation models of colorectal cancer and metastasis. Nat. Biotechnol. 35, 569–576.
Sacchetti, A., Teeuwssen, M., Verhagen, M., Joosten, R., Xu, T., Stabile, R., van der Steen, B., Watson, M.M., Gusinac, A., Kim, W.K., Ubink, I., Van de Werken, H.J.G., Fumagalli, A., Paauwe, M., Van Rheenen, J., Sansom, O.J., Kranenburg, O., Fodde, R., 2021. Phenotypic plasticity underlies local invasion and distant metastasis in colon cancer. eLife 10, e61461.
Sakai, E., Nakayama, M., Oshima, H., Kouyama, Y., Niida, A., Fujii, S., Ochiai, A., Nakayama, K.I., Mimori, K., Suzuki, Y., Hong, C.P., Ock, C.-Y., Kim, S.-J., Oshima, M., 2018. Combined mutation of Apc, Kras, and Tgfbr2 effectively drives metastasis of intestinal cancer. Cancer Res. 78, 1334–1346.
Sakamoto, N., Feng, Y., Stolfi, C., Kurosu, Y., Green, M., Lin, J., Green, M.E., Sentani, K., Yasui, W., McMahon, M., Hardiman, K.M., Spence, J.R., Horita, N., Greenson, J.K., Kuick, R., Cho, K.R., Fearon, E.R., 2017. BRAFV600E cooperates with CDX2 inactivation to promote serrated colorectal tumorigenesis. eLife 6, e20331.
Sato, T., Stange, D.E., Ferrante, M., Vries, R.G., Van Es, J.H., Van Den Brink, S., Van Houdt, W.J., Pronk, A., Van Gorp, J., Siersema, P.D., 2011. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology 141, 1762–1772.
Sato, T., Vries, R.G., Snippert, H.J., van de Wetering, M., Barker, N., Stange, D.E., van Es, J.H., Abo, A., Kujala, P., Peters, P.J., Clevers, H., 2009. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 459, 262–265.
Schutgens, F., Clevers, H., 2020. Human Organoids: Tools for Understanding Biology and Treating Diseases. In: Abbas, A.K., Aster, J.C., Feany, M.B. (Eds.), Annual Review of Pathology: Mechanisms of Disease, 15, pp. 211–234, 2020.
Shin, A.E., Giancotti, F.G., Rustgi, A.K., 2023. Metastatic colorectal cancer: mechanisms and emerging therapeutics. Trends Pharmacol. Sci. 44, 222–236.
Shoemaker, R.H., 2006. The NCI60 human tumour cell line anticancer drug screen. Nat. Rev. Cancer 6, 813–823.
Shultz, L.D., Lyons, B.L., Burzenski, L.M., Gott, B., Chen, X., Chaleff, S., Kotb, M., Gillies, S.D., King, M., Mangada, J., Greiner, D.L., Handgretinger, R., 2005. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. J. Immunol. 174, 6477–6489.
Sid, B., Sartelet, H., Bellon, G., El Btaouri, H., Rath, G., Delorme, N., Haye, B., Martiny, L., 2004. Thrombospondin 1: a multifunctional protein implicated in the regulation of tumor growth. Crit. Rev. Oncol. /Hematol. 49, 245–258.
de Sousa, E.M.F., Harnoss, J.M., Kljavin, N., Scott, R., Sohn, C., Leong, K.G., de Sauvage, F.J., 2020. Modeling colorectal cancer progression through orthotopic implantation of organoids. Methods Mol. Biol. 2171, 331–346.
de Sousa e Melo, F., Kurtova, A.V., Harnoss, J.M., Kljavin, N., Hoeck, J.D., Hung, J., Anderson, J.E., Storm, E.E., Modrusan, Z., Koeppen, H., Dijkgraaf, G.J.P., Piskol, R., de Sauvage, F.J., 2017. A distinct role for Lgr5+ stem cells in primary and metastatic colon cancer. Nature 543, 676–680.
Spence, J.R., Mayhew, C.N., Rankin, S.A., Kuhar, M.F., Vallance, J.E., Tolle, K., Hoskins, E.E., Kalinichenko, V.V., Wells, S.I., Zorn, A.M., Shroyer, N.F., Wells, J.M.,

2011. Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature 470, 105–109.
Strating, E., Verhagen, M.P., Wensink, E., Dünnebach, E., Wijler, L., Aranguren, I., De la Cruz, A.S., Peters, N.A., Hageman, J.H., van der Net, M.M.C., van Schelven, S., Laoukili, J., Fodde, R., Roodhart, J., Nierkens, S., Snippert, H., Gloerich, M., Rinkes, I.B., Elias, S.G., Kranenburg, O., 2023. Co-cultures of colon cancer cells and cancer-associated fibroblasts recapitulate the aggressive features of mesenchymal-like colon cancer. Front Immunol. 14, 1053920.
Stribbling, S.M., Beach, C., Ryan, A.J., 2024. Orthotopic and metastatic tumour models in preclinical cancer research. Pharmacol. Ther. 257, 108631.
Subtil, B., Iyer, K.K., Poel, D., Bakkerus, L., Gorris, M.A.J., Escalona, J.C., van den Dries, K., Cambi, A., Verheul, H.M.W., de Vries, I.J.M., Tauriello, D.V.F., 2023. Dendritic cell phenotype and function in a 3D co-culture model of patient-derived metastatic colorectal cancer organoids. Front Immunol. 14, 1105244.
Szylberg, Ł., Janiczek, M., Popiel, A., Marszałek, A., 2015. Serrated polyps and their alternative pathway to the colorectal cancer: a systematic review. Gastroenterol. Res. Pract. 2015, 573814.
Taraborrelli, L., Şenbabaoğlu, Y., Wang, L., Lim, J., Blake, K., Kljavin, N., Gierke, S., Scherl, A., Ziai, J., McNamara, E., Owyong, M., Rao, S., Calviello, A.K., Oreper, D., Jhunjhunwala, S., Argiles, G., Bendell, J., Kim, T.W., Ciardiello, F., Wongchenko, M. J., de Sauvage, F.J., de Sousa e Melo, F., Yan, Y., West, N.R., Murthy, A., 2023. Tumor-intrinsic expression of the autophagy gene Atg16l1 suppresses anti-tumor immunity in colorectal cancer. Nat. Commun. 14, 5945.
Tauriello, D.V.F., Palomo-Ponce, S., Stork, D., Berenguer-Llergo, A., Badia-Ramentol, J., Iglesias, M., Sevillano, M., Ibiza, S., Cañellas, A., Hernando-Momblona, X., Byrom, D., Matarin, J.A., Calon, A., Rivas, E.I., Nebreda, A.R., Riera, A., Attolini, C. S.-O., Batlle, E., 2018. TGFβ drives immune evasion in genetically reconstituted colon cancer metastasis. Nature 554, 538–543.
Tong, K., Kothari, O.A., Haro, K.S., Panda, A., Bandari, M.M., Carrick, J.N., Hur, J.J., Zhang, L., Chan, C.S., Xing, J., Gatza, M.L., Ganesan, S., Verzi, M.P., 2021. SMAD4 is critical in suppression of BRAF-V600E serrated tumorigenesis. Oncogene 40, 6034–6048.
Toshimitsu, K., Takano, A., Fujii, M., Togasaki, K., Matano, M., Takahashi, S., Kanai, T., Sato, T., 2022. Organoid screening reveals epigenetic vulnerabilities in human colorectal cancer. Nat. Chem. Biol. 18, 605–614.
Utsumi, T., Yamada, Y., Diaz-Meco, M.T., Moscat, J., Nakanishi, Y., 2023. Sessile serrated lesions with dysplasia: is it possible to nip them in the bud? J. Gastroenterol. 58, 705–717.
Varga, J., Nicolas, A., Petrocelli, V., Pesic, M., Mahmoud, A., Michels, B.E., Etlioglu, E., Yepes, D., Häupl, B., Ziegler, P.K., Bankov, K., Wild, P.J., Wanninger, S., Medyouf, H., Farin, H.F., Tejpar, S., Oellerich, T., Ruland, J., Siebel, C.W., Greten, F. R., 2020. AKT-dependent NOTCH3 activation drives tumor progression in a model of mesenchymal colorectal cancer. J. Exp. Med. 217.
Veglia, F., Sanseviero, E., Gabrilovich, D.I., 2021. Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. Nat. Rev. Immunol. 21, 485–498.
de Visser, K.E., Joyce, J.A., 2023. The evolving tumor microenvironment from cancer initiation to metastatic outgrowth. Cancer Cell 41, 374–403.
Wang, M., Bronte, V., Chen, P.W., Gritz, L., Panicali, D., Rosenberg, S.A., Restifo, N.P., 1995. Active immunotherapy of cancer with a nonreplicating recombinant fowlpox virus encoding a model tumor-associated antigen. J. Immunol. 154, 4685–4692.
Weng, J., Li, S., Zhu, Z., Liu, Q., Zhang, R., Yang, Y., Li, X., 2022. Exploring immunotherapy in colorectal cancer. J. Hematol. Oncol. 15, 95.
Westcott, P.M.K., Sacks, N.J., Schenkel, J.M., Ely, Z.A., Smith, O., Hauck, H., Jaeger, A. M., Zhang, D., Backlund, C.M., Beytagh, M.C., Patten, J.J., Elbashir, R., Eng, G., Irvine, D.J., Yilmaz, O.H., Jacks, T., 2021. Low neoantigen expression and poor T-cell priming underlie early immune escape in colorectal cancer. Nat. Cancer 2, 1071–1085.
Yao, Y., Xu, X., Yang, L., Zhu, J., Wan, J., Shen, L., Xia, F., Fu, G., Deng, Y., Pan, M., Guo, Q., Gao, X., Li, Y., Rao, X., Zhou, Y., Liang, L., Wang, Y., Zhang, J., Zhang, H., Li, G., Zhang, L., Peng, J., Cai, S., Hu, C., Gao, J., Clevers, H., Zhang, Z., Hua, G., 2020. Patient-derived organoids predict chemoradiation responses of locally advanced rectal cancer. Cell Stem Cell 26, 17–26 e16.
Yoshida, G.J., 2020. Applications of patient-derived tumor xenograft models and tumor organoids. J. Hematol. Oncol. 13, 4.
Yui, S., Nakamura, T., Sato, T., Nemoto, Y., Mizutani, T., Zheng, X., Ichinose, S., Nagaishi, T., Okamoto, R., Tsuchiya, K., 2012. Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5+ stem cell. Nat. Med. 18, 618–623.
Zhang, L., Adileh, M., Martin, M.L., Klingler, S., White, J., Ma, X., Howe, L.R., Brown, A. M., Kolesnick, R., 2017. Establishing estrogen-responsive mouse mammary organoids from single Lgr5+ cells. Cell. Signal. 29, 41–51.