Published in final edited form as:

Curr Colorectal Cancer Rep. 2018 April ; 14(2): 69–79. doi:10.1007/s11888-018-0403-z.

The Molecular Basis of Metastatic Colorectal Cancer

Sarah F. Andres, PhD, Kathy N. Williams, MS, and Anil K. Rustgi, MD

Division of Gastroenterology, Department of Medicine, Abramson Cancer Center, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

Abstract

Purpose of review—Metastatic colorectal cancer (CRC) is a vexing clinical problem. In contrast to early stage disease, once CRC metastasizes to other organs, long-term survival is compromised. We seek to review the molecular pathogenesis, animal models, and functional genomics for an enhanced understanding of how CRC metastasizes and how this can be exploited therapeutically.

Recent findings—Mouse models may recapitulate certain aspects of metastatic human CRC and allow for studies to identify regulators of metastasis. Modulation of transcription factors, onco-proteins, or tumor suppressors have been identified to activate known metastatic pathways. CD44 variants, microRNAs and RNA binding proteins are emerging as metastatic modulators.

Summary—CRC metastasis is a multi-faceted and heterogeneous disease. Despite common pathways contributing to metastatic development, there are numerous variables that modulate metastatic signals in subsets of patients. It is paramount that studies continue to investigate metastatic drivers, enhancers and inhibitors in CRC to develop therapeutic targets and improve disease outcomes.

Keywords

signaling pathways; WNT TGF β ; HGF; CD44; KRAS

INTRODUCTION

There are approximately 140,000 new cases of colorectal cancer (CRC) in the United States and an estimated 1.4 million cases annually worldwide [1]. Despite significant advances in the prevention, screening and surveillance of CRC in the United States, annual mortality has remained relatively constant at about 50,000 deaths/year, and thus, CRC is one of the leading causes of cancer-related mortality [1]. In CRC, mortality is related typically to metastasis to distant organs, far exceeding mortality that is the consequence of primary

Address correspondence to: Sarah F. Andres, PhD, Division of Gastroenterology, 9 BRB, University of Pennsylvania Perelman School of Medicine, 421 Curie Blvd., Philadelphia, PA 19104, Phone: 215-898-0154, FAX: 215-573-2024, saandres@pennmedicine.upenn.edu.

Compliance with Ethics Guidelines

Conflict of Interest

Sarah F. Andres, Kathy N. Williams, and Anil K. Rustgi declare they have no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

disease. Five-year survival rates are favorable for stages I and II [2]. However, stage IV CRC is beset by challenges in early detection and therapy.

Metastasis formation is a multi-step process involving invasion of primary tumor cells through the basement membrane and intravasation into nearby lymphatics or blood vessels. These tumor cells must then survive transport to distant organ sites (typically liver in CRC) where they extravasate and colonize the secondary organ site to establish micro-metastases. Micro-metastases proliferate to expand at the secondary site, albeit depending upon the tumor type, there may be periods of dormancy. There is a compelling need to illuminate and amplify our understanding of the molecular mechanisms underlying metastatic CRC and to translate this knowledge into new perspectives on prognosis, risk stratification and therapy.

This review covers the salient features of animal models and key signaling pathways that have been implicated in metastatic CRC with a focus on *in vivo* model systems whenever feasible. However, we appreciate that this review will not be able to cover all topics in metastatic CRC, especially as they relate to current therapies.

Mouse models of metastasis

There are a variety of mouse models of CRC metastasis, including xenograft models where tumor cells are injected into the circulation or implanted into the cecum or colon, as well as genetically engineered mouse models (GEMMs), which carry a number of conditional genetic mutations that, when induced, result in metastasis formation [3, *4]. Xenograft models are often immune-compromised, which allows for experimentation with human cells, but eliminates the immune system component from disease progression. Direct injection of tumor cells into the cecal or colonic wall or into the circulation also bypasses some or many of the steps involved in metastasis formation. GEMMs can recapitulate the progression from adenoma to metastatic cancer; however they are expensive and can have long latency.

Implantable mouse models have been long-standing in cancer biology, let alone in CRC. These involve xenografts in which human CRC lines, patient tumor cells [**5], or patient tumor tissue [6] are administered subcutaneously, orthotopically [7] (e.g. in the cecum or distal colon), intrasplenically, or via the portal or tail vein. The latter approaches lend themselves to investigate potential metastasis to the liver and lung, respectively. Preconditioning mice with subcutaneous injection of human CRC cell lines [8] or serial injection and re-derivation [9] may enhance metastasis of CRC cell lines implanted into the cecum or spleen. Recently, the methodology that involves cecal [10, *4] or colonic [**11- **13] implantation of 3D CRC organoids has been described, which allows for *in vitro* manipulation followed by *in vivo* transplantation. The ability to use mouse cell lines in syngeneic models offers advantages of an intact immune system and evaluation of the tumor microenvironment [**11, **12]. Using patient derived tumor xenograft (PDX) models more closely approximates human disease and mirrors clinical outcomes [14, **11]. Furthermore, humanized mouse models may offer new opportunities in CRC [15].

Genetic mouse models of CRC involve manipulation of key oncogenes (e.g. mutant *Kras*, mutant *Braf*) and tumor suppressor genes (*Apc*, *Trp53*, *Tgfbr2/Smad4*). One principle

appears to be that activation of individual oncogenes or inactivation of tumor suppressor genes is insufficient to yield metastasis. For example, neither mutant *Kras* nor mutant *Braf* can promote substantial primary tumorigenesis let alone metastatic tumors [16]. These may be due in part to oncogene mediated senescence or lack of expansion of mutant stem cell clones that require additional genetic alterations to overcome these barriers [17, 18]. To that end, deletion of *Ink4a/Arf* in *K-ras^{G12D}* mice surmounts senescence and leads to invasive and metastatic cancers [19] and oncogenic *Braf^{V600E}* mutation coupled with microsatellite instability, *p16^{Ink4a}* inactivation, and *Tip53* mutation results in the emergence of primary and metastatic tumors [20, 21]. Interestingly, these two genetic models share the development and characterization of the sessile serrated adenoma pathway to CRC.

Other examples of phenocopying metastatic CRC (Table 1) involve activation of the Wnt signaling pathway and of *Kras* along with inactivation of *Tgfr2* [*22-24] or employing inducible mutant *Kras* and conditional null alleles of *Apc* and *Tip53* [*25]. *Apc^{CKO/CKO}-Kras^{LSL-G12D/+}* mice develop metastatic lesions to the liver after 6 months [26]. Herein, these approaches are reminiscent of the chromosomal instability pathway that underlies the majority of sporadic CRC.

Interestingly, conditional knockout of mismatch repair genes, either individually or in combination, have not been reported to result in metastatic CRC [27]; although orthotopic injection of tumor cells from a patient with microsatellite instability (MSI) into immune-deficient mice did result in liver metastasis formation [*12], presumably due to the missing immune component. Of note, in humans, high microsatellite instability (MSI-H) colorectal tumors represent about 15% of all sporadic colorectal tumors and such tumors have better prognosis when compared to their microsatellite stable counterparts at the same stage.

Very recently the use of orthotopic organoid transplantation directly into the colonic mucosa or cecal wall of immune-deficient or syngeneic mouse models [*10, *13, *12] or administration of organoid fragments into the colon by enema [11] has emerged as a method for studying CRC progression and metastasis (Table 2). These systems combine conditional genetic alleles, CRISPR/Cas9 technology, and lentiviral systems to manipulate mouse or human colonic organoids *ex vivo* before transplantation into the animal model of choice [*10, *13, *11, *12, *4]. These systems, as well as the use of directed adenovirus injection [*12] or localized tamoxifen administration [*25], allow for highly specific targeting of colonic cells to induce expression of mutant oncogenes or knockout tumor suppressors and improve potentially upon current CRC models.

Signaling pathways that drive or enhance CRC metastasis

WNT/ β -catenin—Certainly, the afore-mentioned mouse models underscore the importance of activated WNT signaling in both colorectal tumor initiation and tumor metastasis. In this context, nuclear translocation and accumulation of β -catenin is important, with resulting induction of numerous gene targets that foster proliferation and growth. BCL9-2, a β -catenin binding protein, may be part of a regulatory network that modulates β -catenin's cellular adhesive properties versus transcriptional functions [28]. Studies reveal variable nuclear β -catenin in both primary and metastatic colorectal tumors, with more prominent accumulation in invasive tumor fronts, thereby reflecting tumor heterogeneity [29] and cell stemness [30].

WNT/ β -catenin signaling is central to a number of pathways that contribute to the metastatic progression of CRC. For example, DACT2 is a tumor suppressor that directly inhibits WNT/ β -catenin signaling and when lost enhances metastasis [31]. The transcription factor PROX1 is downstream of WNT and promotes survival of WNT-high tumor-initiating cells in metabolically stressed environments. Targeting PROX1 or downstream effectors are viable therapeutic options for patients who have developed anti-EGFR therapy resistance, but have PROX1 positivity [32]. The GTPase RhoA acts upstream of β -catenin to maintain membranous localization. Loss of RhoA leads to accumulation of nuclear β -catenin and enhances metastasis formation in xenograft mouse models [33]. Identifying patients with nuclear β -catenin levels is critical to determining effective therapy options, as use of the AKT inhibitor (API-2) has been shown to promote cell survival and metastasis in PDX models through synergistic interactions with nuclear β -catenin and FOXO3a [34]. While inhibitors of WNT signaling are used in preclinical models, they are not yet advanced in clinical trials.

TGF- β pathway—This pathway and its principle components—receptors types I and II, SMAD3 and SMAD4, are altered in different stages of CRC. Mutations in TGF- β type II receptor are associated with microsatellite instability [35]. Given the pleiotropic effects of TGF- β signaling in normal intestinal epithelial homeostasis and in malignant cells, it should be noted that these effects are cell autonomous and non-cell autonomous. One striking finding is that TGF- β signaling affects stromal cells to “prime” metastatic colonization by CRC cells [36]. This is mediated by secretion of IL-11 from cancer-associated fibroblasts stimulated by TGF- β with resulting GP130/STAT3 signaling in the tumor cells. Pharmacological inhibition of TGF- β signaling limited metastasis formation [36].

The role of epithelial TGF- β in metastasis is not clear. Several studies suggest that TGF- β signaling is protective against metastatic CRC [37, *22, 38]. Conversely, others have shown that TGF- β promotes metastatic spread downstream of mutant KRAS [25], suggesting that caution must be exercised if targeting TGF- β to prevent metastatic spread. Neutralizing antibodies targeting TGF- β ligand(s) or inhibitors of components of the TGF- β pathway are conceptually appealing and remain investigational, largely speaking.

KRAS/BRAF—*KRAS* mutations are a common hallmark of CRC progression, where the mutated GTPase is permanently stuck in an activated state [39]. Although dispensable for primary tumor growth [25, 26, *22], mutant *Kras* appears to be a strong driver of invasion and metastasis [19, 25, 26, *22]. The combination of mutant *Kras* with other genetic alterations is evidenced also when combined *Tgfr2* resulting in metastatic tumors to the lymph nodes and lungs [24]. An intrasplenic injection xenograft model demonstrated that ERK2 activation (in a mutant *Kras* background) specifically facilitates liver metastases, while inhibition of p38/MAPK signaling specifically enhances lung metastases [40]. Loss of p38 promotes secretion of PTHLH, which causes caspase-independent apoptosis of the lung microvasculature, facilitating extravasation of the tumor cells into the lung tissue [40].

BRAF is one of three protein kinases in the MAPK signaling pathway [41]. Mutations in *BRAF* are strongly associated with poor prognosis [41], and are prevalent in patients with high microsatellite instability (MSI-H). Animal models demonstrate that selective

expression of mutant *Braf* in the intestinal and colonic epithelium does promote tumor formation with some lymph node involvement [20]. Mutant *Braf* combined with either a *Trp53* or *Cdkn2a* (p16) mutation greatly enhances metastatic spread [20]. There are several therapeutic options for patients with BRAF mutations, including combinations of conventional chemotherapeutics [41]. Recent studies have focused on therapies combining BRAF and EGFR inhibitors with MEK, PI3K, or WNT inhibition [41].

Hepatocyte growth factor (HGF)/MET—The hepatocyte growth factor (HGF) acts via binding its cognate receptor tyrosine kinase, MET, which is critical to embryonic development and hyper-activated in primary CRC tumor development and metastasis [42]. MET is a major signaling node that when activated promotes CRC metastasis. Recent studies have identified a number of transcription factors that directly facilitate MET expression, promote metastasis in *in vivo* models, and whose expression is elevated in metastatic patient samples. FOXC2 directly targets MET to promote metastasis *in vivo* [43]. The transcription factor metastasis-associated in colon cancer-1 (MACC1) [44] directly binds to the MET promoter to enhance transcription in an HGF-dependent manner. Several studies have demonstrated that targeting MACC1 genetically or chemically reduces metastases in animal models [45, 46]. Interestingly, lovastatin and rottlerin are small-molecule inhibitors of MACC1 [46]. These are FDA approved compounds, which could be repositioned for use in metastatic CRC patients. Although patients who use statins have a reduced risk of developing CRC, statins have shown poor efficacy in the clinic for treating CRC. Perhaps stratifying patients based on MACC1 expression will improve clinical outcomes for this subset of patients [46].

Signaling through MET is not only controlled by receptor expression but also by structural components that facilitate HGF ligand binding. CD44 is an alternatively spliced, transmembrane glycoprotein that is highly expressed in intestinal stem cells and adenomas and acts as a co-receptor for receptor tyrosine kinases, as well as other signaling molecules (reviewed by [47]). Enhanced splicing variation is associated with tumor aggressiveness in CRC patients [48] and CD44 variant expression promotes adenoma formation in *Apc^{Min/+}* mice [49, 50]. CD44 variants and MET together promote the growth of *ex vivo* adenoma organoid cultures and the variant CD44v6 is required for HGF/MET signaling [51, 52]. It was shown that patient metastases were enriched for cells expressing CD44v6 and that these cells were responsible for metastasis formation in mice. CD44v6 expression is stimulated by microenvironmental signals, including HGF, from cancer-associated fibroblasts. Blocking CD44v6 or MET limited migration *in vitro* and reduced metastatic spread of patient-derived tumor cells in animal models [**5]. CD44v6 cooperates with MET to respond to signals from the microenvironment, activate PI3K/AKT signaling and promote cellular survival, migration, and ultimately metastasis [**5]. This work illustrates that CD44v6 is one component of a complex metastatic signaling profile that confers metastatic potential to select cell populations within the primary tumor [**5]. CD44 is not only involved in HGF/MET signaling, but is also upstream of a number of other oncogenic mediators, including cMyc and STAT3, which promote metastasis of CRC cells via anoikis resistance and enhancing EMT properties [53].

There are a number of therapeutic approaches that have been developed to target CD44 variants, including methods that disrupt binding to hyaluronic acid (antibodies, soluble competitive ectodomains, or nanoparticle-infused chemotherapeutic delivery), monoclonal antibodies, aptamers that inhibit binding functions, blocking peptides, and methods for blocking CD44 expression, including si- and shRNA delivery (reviewed by [47]). Future studies could focus on identifying and targeting isoform- and tumor-specific CD44-mediated effects.

PI3K/AKT/mTOR—The PI3K/AKT pathway propagates signals from growth factors, cytokines, and oncogenic-mediators to contribute to CRC pathogenesis. The tumor suppressor PTEN inhibits PI3K/AKT signaling. Loss of PTEN promotes metastasis in CRC [54] as does expression of the adaptor protein paxillin [55], both of which activate PI3K/AKT signaling. AKT2, which is highly expressed in late stage CRC, is essential for establishment of liver metastases and when combined with the loss of PTEN further enhances metastasis in the intrasplenic injection model [56]. IL13 also induces PI3K/AKT and signal propagation relies in part on the scaffolding protein FAM120A. FAM120A stabilizes multi-protein complexes allowing for transmission of metastatic signals [57]. Loss of FAM120A alone or in combination with IL13Ra2 inhibition enhanced the survival of mice in an intrasplenic injection model of liver metastasis [57]. Targeting PI3K itself or the downstream effector mTOR has proven effective for reducing metastasis in animal models [58, 59]. Previous attempts to use rapamycin to target mTORC1 in CRC patients have not been fruitful; however, dual targeting of mTORC1 and 2 may yield more favorable outcomes [59]. Combination targeting of mTOR and other molecules, such as stromal target PDGF, may also improve outcomes [60].

Epidermal growth factor receptor (EGFR)—While the epidermal growth factor receptor (EGFR) is a known contributor to the development of primary CRC [61] and is targeted therapeutically with antibodies or small molecule inhibitors [62], there is a lack of model systems that link EGFR signaling directly to CRC metastasis. A variety of studies provide correlative evidence for EGFR expression in advanced [63] or metastatic CRC [64]. EGFR expression is correlated with worse prognosis [65]. Enhanced EGFR signaling may partially contribute to lung metastasis in a mouse model of metastatic colon cancer (*VilCre;Tgfr2^{fl/f};Kras^{LSL-G12D/+}*) [24]. Since EGFR expression does not necessarily predict response to therapy [66], it is possible and likely that EGFR signaling in non-epithelial tissues is contributing to tumor progression. Recent work has demonstrated that EGFR expression on myeloid cells promoted tumor growth in a mouse model of inflammation-associated cancer [67]. The detection EGFR on circulating extracellular vesicles isolated from the blood of xenograft mice bearing CRC cell line tumors is an example of the ongoing optimization of liquid biopsy methods for early CRC detection and monitoring [68].

Global metastasis modulators: microRNAs and RNA binding proteins

miRNAs—microRNAs (miRNAs) are important regulators of gene expression as they modulate multiple targets within a signaling pathway simultaneously. The processing and maturation of miRNAs is dependent upon the activity of the enzyme DICER and modulations in DICER expression and/or activity alter the available miRNA pool. Loss of

DICER promotes metastasis in many tissue types, including from the colon [69, 70]. Loss of DICER enhances a tumor-initiating cell population, increases markers of epithelial to mesenchymal transition, and promotes metastasis via downregulation of tumor suppressor miRNAs (miR-126, miR-34a, and miR-335) [69].

While the body of literature on miRNAs and CRC is extensive, we will highlight certain miRNAs (miR-34a and miR15a/16-1) that have been validated in animal models as playing direct roles in CRC metastasis. The miR-34a is a tumor suppressor that acts downstream of the IL-6/STAT3 pathway to inhibit epithelial to mesenchymal transition, invasion, and metastasis. IL-6 represses miR-34a directly via STAT3 and treating CRC cells with IL-6 enhances lung metastasis in a tail vein injection model [71]. Loss of STAT3 or IL-6R inhibits metastasis and loss of miR-34a promotes invasion in the dextran-sodium sulfate mouse model of inflammation-associated CRC [71]. In humans, silencing of miR-34a has been associated with lymph node involvement and distant metastases in human patient samples [72]. Administration of miR-34a to mice inhibited metastasis of prostate cancer cells to the lung, in part through direct targeting of CD44 [73].

miR-15a/16-1 are also tumor suppressive miRNAs that inhibit transcription of AP4 [74]. AP4 has been previously shown to promote metastasis [75] and ectopic expression of miR-15a/16-1 limits AP4-mediated metastasis in the tail vein injection model [74].

mRNA binding proteins—Like miRNAs, RNA binding proteins (RBPs) can also regulate entire signaling pathways. RBPs can modulate the location, maturation, stability, and/or translation of target mRNAs [76]. We have identified LIN28B, a pluripotency factor and inhibitor of miRNA *let-7* biogenesis, as a promoter of metastasis in CRC. Using a variety of human CRC cell lines we demonstrated that LIN28B expression promoted metastasis *in vivo* in the subcutaneous xenograft model where metastases are less common [77]. Additionally, we have shown that expression of the RNA binding protein insulin-like growth factor 2 mRNA binding protein 1 (IMP1) is significantly increased in lymph node involved tissues from CRC patients [78], suggesting a role for IMP1 in CRC metastasis. IMP1 expression correlates with poor prognosis, reduced survival and increased metastasis in CRC patient samples and promotes a metastatic phenotype *in vitro* [79, 80]. Expression of another IMP isoform, IMP3, correlates with worse prognosis, reduced survival, and increased metastasis in CRC [81, 82], while expression of ELAV-like protein 1 (HUR) expression is correlated with poor survival in CRC, but not metastasis specifically [83].

The Musashi family of RNA binding proteins is required for intestinal tumorigenesis [84] and plays important roles in CRC tumor formation and progression by targeting a number of key signaling pathways, including Notch [85], Wnt [85], PTEN/mTOR [86, 84], TGF- β /SMAD3, Myc, and MET [87, 86]. Similar to IMP1, IMP3, and HUR, Musashi-1 and -2 expression is also highly correlated with poor prognosis, reduced disease-free survival, lymph node involvement, and liver metastases in CRC patients [88, 89]; however there are no studies that examine the direct impact of Musashi on CRC metastasis.

A closer examination of direct roles for these RNA binding proteins in CRC metastasis is warranted, as targeting an RNA binding protein could silence oncogenic signaling at

multiple points, rather than targeting a single protein in a given pathway. For example, studies demonstrate that IMP1 targets KRAS [90, 91], CD44 [90, 92], and other oncogenic mediators suggesting that targeting IMP1 in certain circumstances may also be beneficial to limiting CRC progression. Recent work has identified an IMP1 inhibitor in ovarian and melanoma cells [93] and described IMP1 inhibition as enhancing treatment efficacy in BRAF^{V600E} melanoma [94]. Targeting Musashi could simultaneously shutdown metastatic signals through Notch, WNT, mTOR, TGF- β , myc, and MET. Efforts to identify Musashi inhibitors are underway and demonstrate its utility as a target; however, candidate approaches are still in the discovery stage or animal model testing (reviewed in [87]).

Conclusion

Recent work demonstrates a growing understanding of molecular drivers and enhancers of metastasis in CRC (Figure 1). Current efforts on GEMMs, humanized animal models with patient-derived tumor cells, 3D organoids, identification of new biomarkers, and testing of potential therapeutics will advance the field. Metastases typically result from the culmination of many aberrant signaling events rather than a single mutation or abnormal protein and this progression is highly variable between patients. Focusing the development of therapeutics on targeting broad metastatic regulators, such as miRNAs or mRNA binding proteins may have a greater impact than targeting a single protein within a signaling cascade. Developing diagnostic and prognostic markers from liquid biopsy or from markers of metastatic potential within the primary tumor may allow for personalized medicine. Using patient derived tumor cells, rather than cell lines, to test therapies should also improve our ability to rapidly identify and validate new targets.

Acknowledgments

The authors were supported by the NIH/NIDDK P30DK050306, R01DK056645, F32 DK107052 (SFA), AGA Research Scholar Award (SFA), HHMI Research Fellowship (KNW), Hansen Foundation, Lustgarten Family colon cancer grants, and the Penn Colon Cancer Translational Center of Excellence.

References

1. Cancer Facts & Figures [database on the Internet]. American Cancer Society; 2018. Accessed:
2. Health NIO. National Cancer Institute. 2018. <https://www.cancer.gov/>. Accessed 1/25/2018 2018.
3. Oh BY, Hong HK, Lee WY, Cho YB. Animal models of colorectal cancer with liver metastasis. *Cancer Lett.* 2017; 387:114–20. DOI: 10.1016/j.canlet.2016.01.048 [PubMed: 26850374]
4. Fumagalli A, Suijkerbuijk SJE, Begthel H, Beerling E, Oost KC, Snippert HJ, et al. A surgical orthotopic organoid transplantation approach in mice to visualize and study colorectal cancer progression. *Nat Protoc.* 2018; 13(2):235–47. This methods paper describes a new model of metastasis using organoids derived from mouse or human patient tumors. The generation of these organoids allows for ex vivo genetic manipulation following orthotopic transplantation into the cecum of mice. These tumor organoids exhibit metastatic spread that closely mimics human disease and they can be observed in real time by intravital imaging. DOI: 10.1038/nprot.2017.137 [PubMed: 29300390]
5. Todaro M, Gaggianesi M, Catalano V, Benfante A, Iovino F, Biffoni M, et al. CD44v6 is a marker of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis. *Cell Stem Cell.* 2014; 14(3):342–56. This study exemplifies the use of patient-derived tumor cells in metastatic animal models. Herein they demonstrate a critical role for CD44v6+ cells in driving metastatic spread in CRC. They also show the importance of tumor-associated fibroblasts signals

in simulating these metastatic tumor initiators. DOI: 10.1016/j.stem.2014.01.009 [PubMed: 24607406]

6. Sun FX, Sasson AR, Jiang P, An Z, Gamagami R, Li L, et al. An ultra-metastatic model of human colon cancer in nude mice. *Clin Exp Metastasis*. 1999; 17(1):41–8. [PubMed: 10390146]
7. Kashtan H, Rabau M, Mullen JB, Wong AH, Roder JC, Shpitz B, et al. Intra-rectal injection of tumour cells: a novel animal model of rectal cancer. *Surg Oncol*. 1992; 1(3):251–6. [PubMed: 1341258]
8. Alamo P, Gallardo A, Di Nicolantonio F, Pavon MA, Casanova I, Trias M, et al. Higher metastatic efficiency of KRas G12V than KRas G13D in a colorectal cancer model. *FASEB J*. 2015; 29(2): 464–76. DOI: 10.1096/fj.14-262303 [PubMed: 25359494]
9. Morikawa K, Walker SM, Nakajima M, Pathak S, Jessup JM, Fidler IJ. Influence of organ environment on the growth, selection, and metastasis of human colon carcinoma cells in nude mice. *Cancer Res*. 1988; 48(23):6863–71. [PubMed: 2846163]
- 10••. Fumagalli A, Drost J, Suijkerbuijk SJ, van Boxtel R, de Ligt J, Offerhaus GJ, et al. Genetic dissection of colorectal cancer progression by orthotopic transplantation of engineered cancer organoids. *Proc Natl Acad Sci U S A*. 2017; 114(12):E2357–E64. Utilized human organoids genetically modified with CRISPR/Cas9 to contain combinations of APC, P53, KRAS, and SMAD4 deletions or mutations. These were transplanted into the cecal wall of immune-deficient mice to examine roles for each genetic combination in tumor formation and metastasis. DOI: 10.1073/pnas.1701219114 [PubMed: 28270604]
- 11••. O'Rourke KP, Loizou E, Livshits G, Schatoff EM, Baslan T, Manchado E, et al. Transplantation of engineered organoids enables rapid generation of metastatic mouse models of colorectal cancer. *Nat Biotechnol*. 2017; 35(6):577–82. Utilized novel method of organoid fragment delivery to the mouse colon via enema for rapid generation of colonic tumors that recapitulate the human adenoma – carcinoma – metastasis malignant progression. System is easily adaptable to mouse into mouse or human into mouse experiments and allows for ex vivo manipulation of organoids prior to transplantation. DOI: 10.1038/nbt.3837 [PubMed: 28459450]
- 12••. Roper J, Tammela T, Cetinbas NM, Akkad A, Roghanian A, Rickelt S, et al. In vivo genome editing and organoid transplantation models of colorectal cancer and metastasis. *Nat Biotechnol*. 2017; 35(6):569–76. Demonstrated colonoscopy-guided mucosal injection of adeno-Cre or lentivirus carrying guides for Apc or Trp53 into the colonic mucosa of mice carrying conditional genetic alleles for Apc or Cas9 resulting in tumor formation. Elegant use of conditional Cre or CRISPR/Cas9 technology in organoids derived from mouse and human sources. These organoids were injected into the colonic mucosa of NSG or humanized mice to model tumor formation and/or metastasis following genetic recombination. DOI: 10.1038/nbt.3836 [PubMed: 28459449]
- 13••. de Sousa e Melo F, Kurtova AV, Harnoss JM, Kljavin N, Hoeck JD, Hung J, et al. A distinct role for Lgr5(+) stem cells in primary and metastatic colon cancer. *Nature*. 2017; 543(7647):676–80. Developed organoids from Lgr5^{DTR-eGFP} mice bearing mutations in Apc, Kras, Trp53, and Smad4, which were generated through a combination of mouse genetics and CRISPR/Cas9 technology. Using these tools they demonstrated that Lgr5+ cells initiate tumor formation and metastasis, but that ablation of Lgr5+ cells within a formed tumor only slows tumor growth and does not lead to tumor regression. DOI: 10.1038/nature21713 [PubMed: 28358093]
14. Nunes M, Vrignaud P, Vacher S, Richon S, Lievre A, Cacheux W, et al. Evaluating patient-derived colorectal cancer xenografts as preclinical models by comparison with patient clinical data. *Cancer Res*. 2015; 75(8):1560–6. DOI: 10.1158/0008-5472.CAN-14-1590 [PubMed: 25712343]
15. Margolin DA, Myers T, Zhang X, Bertoni DM, Reuter BA, Obokhare I, et al. The critical roles of tumor-initiating cells and the lymph node stromal microenvironment in human colorectal cancer extranodal metastasis using a unique humanized orthotopic mouse model. *FASEB J*. 2015; 29(8): 3571–81. DOI: 10.1096/fj.14-268938 [PubMed: 25962655]
16. Feng Y, Bommer GT, Zhao J, Green M, Sands E, Zhai Y, et al. Mutant KRAS promotes hyperplasia and alters differentiation in the colon epithelium but does not expand the presumptive stem cell pool. *Gastroenterology*. 2011; 141(3):1003–13. e1–10. DOI: 10.1053/j.gastro.2011.05.007 [PubMed: 21699772]
17. Janssen KP, Alberici P, Fsihi H, Gaspar C, Breukel C, Franken P, et al. APC and oncogenic KRAS are synergistic in enhancing Wnt signaling in intestinal tumor formation and progression.

- Gastroenterology. 2006; 131(4):1096–109. DOI: 10.1053/j.gastro.2006.08.011 [PubMed: 17030180]
18. Sakamoto N, Feng Y, Stolfi C, Kurosu Y, Green M, Lin J, et al. BRAF(V600E) cooperates with CDX2 inactivation to promote serrated colorectal tumorigenesis. *Elife*. 2017; 6doi: 10.7554/eLife.20331
 19. Bennecke M, Kriegl L, Bajbouj M, Retzlaff K, Robine S, Jung A, et al. Ink4a/Arf and oncogene-induced senescence prevent tumor progression during alternative colorectal tumorigenesis. *Cancer Cell*. 2010; 18(2):135–46. DOI: 10.1016/j.ccr.2010.06.013 [PubMed: 20708155]
 20. Rad R, Cadigan J, Rad L, Varela I, Strong A, Kriegl L, et al. A genetic progression model of Braf(V600E)-induced intestinal tumorigenesis reveals targets for therapeutic intervention. *Cancer Cell*. 2013; 24(1):15–29. DOI: 10.1016/j.ccr.2013.05.014 [PubMed: 23845441]
 21. Rustgi AK. BRAF: a driver of the serrated pathway in colon cancer. *Cancer Cell*. 2013; 24(1):1–2. DOI: 10.1016/j.ccr.2013.06.008 [PubMed: 23845435]
 - 22•. Sakai E, Nakayama M, Oshima H, Kouyama Y, Niida A, Fujii S, et al. Combined mutation of Apc, Kras and Tgfr2 effectively drives metastasis of intestinal cancer. *Cancer Res*. 2017; **This study used compound genetic mouse models and organoids to assess stepwise drivers of primary tumor formation and metastasis *in vivo*. They performed RNA sequencing analysis to identify common changes in gene expression downstream of metastatic drivers.** doi: 10.1158/0008-5472.CAN-17-3303
 23. Munoz NM, Upton M, Rojas A, Washington MK, Lin L, Chytil A, et al. Transforming growth factor beta receptor type II inactivation induces the malignant transformation of intestinal neoplasms initiated by Apc mutation. *Cancer Res*. 2006; 66(20):9837–44. DOI: 10.1158/0008-5472.CAN-06-0890 [PubMed: 17047044]
 24. Trobridge P, Knoblaugh S, Washington MK, Munoz NM, Tsuchiya KD, Rojas A, et al. TGF-beta receptor inactivation and mutant Kras induce intestinal neoplasms in mice via a beta-catenin-independent pathway. *Gastroenterology*. 2009; 136(5):1680–8.e7. DOI: 10.1053/j.gastro.2009.01.066 [PubMed: 19208363]
 - 25••. Boutin AT, Liao WT, Wang M, Hwang SS, Karpinets TV, Cheung H, et al. Oncogenic Kras drives invasion and maintains metastases in colorectal cancer. *Genes Dev*. 2017; 31(4):370–82. Developed a genetic mouse model with colon-specific, inducible Apc and Trp53 loss coupled with mutant Kras expression. These mice recapitulate many aspects of human CRC, including liver metastasis. This study demonstrates the importance of mutant KRAS and TGF-β signaling for invasion and metastasis. DOI: 10.1101/gad.293449.116 [PubMed: 28289141]
 26. Hung KE, Maricevich MA, Richard LG, Chen WY, Richardson MP, Kunin A, et al. Development of a mouse model for sporadic and metastatic colon tumors and its use in assessing drug treatment. *Proc Natl Acad Sci U S A*. 2010; 107(4):1565–70. DOI: 10.1073/pnas.0908682107 [PubMed: 20080688]
 27. Taketo MM, Edelmann W. Mouse models of colon cancer. *Gastroenterology*. 2009; 136(3):780–98. [PubMed: 19263594]
 28. Brembeck FH, Schwarz-Romond T, Bakkers J, Wilhelm S, Hammerschmidt M, Birchmeier W. Essential role of BCL9-2 in the switch between beta-catenin's adhesive and transcriptional functions. *Genes Dev*. 2004; 18(18):2225–30. DOI: 10.1101/gad.317604 [PubMed: 15371335]
 29. Fodde R, Brabletz T. Wnt/beta-catenin signaling in cancer stemness and malignant behavior. *Curr Opin Cell Biol*. 2007; 19(2):150–8. DOI: 10.1016/j.ccb.2007.02.007 [PubMed: 17306971]
 30. Vermeulen L, De Sousa EMF, van der Heijden M, Cameron K, de Jong JH, Borovski T, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol*. 2010; 12(5):468–76. DOI: 10.1038/ncb2048 [PubMed: 20418870]
 31. Wang S, Dong Y, Zhang Y, Wang X, Xu L, Yang S, et al. DACT2 is a functional tumor suppressor through inhibiting Wnt/beta-catenin pathway and associated with poor survival in colon cancer. *Oncogene*. 2015; 34(20):2575–85. DOI: 10.1038/onc.2014.201 [PubMed: 25023701]
 32. Ragusa S, Cheng J, Ivanov KI, Zangger N, Ceteci F, Bernier-Latmani J, et al. PROX1 promotes metabolic adaptation and fuels outgrowth of Wnt(high) metastatic colon cancer cells. *Cell Rep*. 2014; 8(6):1957–73. DOI: 10.1016/j.celrep.2014.08.041 [PubMed: 25242332]

33. Rodrigues P, Macaya I, Bazzocco S, Mazzolini R, Andretta E, Dopeso H, et al. RHOA inactivation enhances Wnt signalling and promotes colorectal cancer. *Nat Commun.* 2014; 5:5458.doi: 10.1038/ncomms6458 [PubMed: 25413277]
34. Tenbaum SP, Ordonez-Moran P, Puig I, Chicote I, Arques O, Landolfi S, et al. beta-catenin confers resistance to PI3K and AKT inhibitors and subverts FOXO3a to promote metastasis in colon cancer. *Nat Med.* 2012; 18(6):892–901. DOI: 10.1038/nm.2772 [PubMed: 22610277]
35. Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, et al. Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science.* 1995; 268(5215): 1336–8. [PubMed: 7761852]
36. Calon A, Espinet E, Palomo-Ponce S, Tauriello DV, Iglesias M, Cespedes MV, et al. Dependency of colorectal cancer on a TGF-beta-driven program in stromal cells for metastasis initiation. *Cancer Cell.* 2012; 22(5):571–84. DOI: 10.1016/j.ccr.2012.08.013 [PubMed: 23153532]
37. Fritzmann J, Morkel M, Besser D, Budczies J, Kosel F, Brembeck FH, et al. A colorectal cancer expression profile that includes transforming growth factor beta inhibitor BAMBI predicts metastatic potential. *Gastroenterology.* 2009; 137(1):165–75. DOI: 10.1053/j.gastro.2009.03.041 [PubMed: 19328798]
38. Itatani Y, Kawada K, Fujishita T, Kakizaki F, Hirai H, Matsumoto T, et al. Loss of SMAD4 from colorectal cancer cells promotes CCL15 expression to recruit CCR1+ myeloid cells and facilitate liver metastasis. *Gastroenterology.* 2013; 145(5):1064–75.e11. DOI: 10.1053/j.gastro.2013.07.033 [PubMed: 23891973]
39. McCormick F. K-Ras protein as a drug target. *J Mol Med (Berl).* 2016; 94(3):253–8. DOI: 10.1007/s00109-016-1382-7 [PubMed: 26960760]
40. Urošević J, Garcia-Albeniz X, Planet E, Real S, Cespedes MV, Guiu M, et al. Colon cancer cells colonize the lung from established liver metastases through p38 MAPK signalling and PTHLH. *Nat Cell Biol.* 2014; 16(7):685–94. DOI: 10.1038/ncb2977 [PubMed: 24880666]
41. Strickler JH, Wu C, Bekaii-Saab T. Targeting BRAF in metastatic colorectal cancer: Maximizing molecular approaches. *Cancer Treat Rev.* 2017; 60:109–19. DOI: 10.1016/j.ctrv.2017.08.006 [PubMed: 28946014]
42. Mazzone M, Comoglio PM. The Met pathway: master switch and drug target in cancer progression. *FASEB J.* 2006; 20(10):1611–21. DOI: 10.1096/fj.06-5947rev [PubMed: 16873884]
43. Cui YM, Jiao HL, Ye YP, Chen CM, Wang JX, Tang N, et al. FOXC2 promotes colorectal cancer metastasis by directly targeting MET. *Oncogene.* 2015; 34(33):4379–90. DOI: 10.1038/nc.2014.368 [PubMed: 25381815]
44. Stein U, Walther W, Arlt F, Schwabe H, Smith J, Fichtner I, et al. MACC1, a newly identified key regulator of HGF-MET signaling, predicts colon cancer metastasis. *Nat Med.* 2009; 15(1):59–67. DOI: 10.1038/nm.1889 [PubMed: 19098908]
45. Pichorner A, Sack U, Kobelt D, Kelch I, Arlt F, Smith J, et al. In vivo imaging of colorectal cancer growth and metastasis by targeting MACC1 with shRNA in xenografted mice. *Clin Exp Metastasis.* 2012; 29(6):573–83. DOI: 10.1007/s10585-012-9472-6 [PubMed: 22484916]
46. Juneja M, Kobelt D, Walther W, Voss C, Smith J, Specker E, et al. Statin and rottlerin small-molecule inhibitors restrict colon cancer progression and metastasis via MACC1. *PLoS Biol.* 2017; 15(6):e2000784.doi: 10.1371/journal.pbio.2000784 [PubMed: 28570591]
47. Orian-Rousseau V, Ponta H. Perspectives of CD44 targeting therapies. *Arch Toxicol.* 2015; 89(1): 3–14. DOI: 10.1007/s00204-014-1424-2 [PubMed: 25472903]
48. Wielenga VJ, Heider KH, Offerhaus GJ, Adolf GR, van den Berg FM, Ponta H, et al. Expression of CD44 variant proteins in human colorectal cancer is related to tumor progression. *Cancer Res.* 1993; 53(20):4754–6. [PubMed: 7691404]
49. Zeilstra J, Joosten SP, Dokter M, Verwiel E, Spaargaren M, Pals ST. Deletion of the WNT target and cancer stem cell marker CD44 in Apc(Min/+) mice attenuates intestinal tumorigenesis. *Cancer Res.* 2008; 68(10):3655–61. DOI: 10.1158/0008-5472.CAN-07-2940 [PubMed: 18483247]
50. Zeilstra J, Joosten SP, van Andel H, Tolg C, Berns A, Snoek M, et al. Stem cell CD44v isoforms promote intestinal cancer formation in Apc(min) mice downstream of Wnt signaling. *Oncogene.* 2014; 33(5):665–70. DOI: 10.1038/nc.2012.611 [PubMed: 23318432]

51. Orian-Rousseau V, Chen L, Sleeman JP, Herrlich P, Ponta H. CD44 is required for two consecutive steps in HGF/c-Met signaling. *Genes Dev.* 2002; 16(23):3074–86. DOI: 10.1101/gad.242602 [PubMed: 12464636]
52. Joosten SPJ, Zeilstra J, van Andel H, Mijns RC, Zaunbrecher J, Duivenvoorden AAM, et al. MET Signaling Mediates Intestinal Crypt-Villus Development, Regeneration, and Adenoma Formation and Is Promoted by Stem Cell CD44 Isoforms. *Gastroenterology.* 2017; 153(4):1040–53 e4. DOI: 10.1053/j.gastro.2017.07.008 [PubMed: 28716720]
53. Su YJ, Lai HM, Chang YW, Chen GY, Lee JL. Direct reprogramming of stem cell properties in colon cancer cells by CD44. *EMBO J.* 2011; 30(15):3186–99. DOI: 10.1038/emboj.2011.211 [PubMed: 21701559]
54. Zhang LL, Mu GG, Ding QS, Li YX, Shi YB, Dai JF, et al. Phosphatase and Tensin Homolog (PTEN) Represses Colon Cancer Progression through Inhibiting Paxillin Transcription via PI3K/AKT/NF-kappaB Pathway. *J Biol Chem.* 2015; 290(24):15018–29. DOI: 10.1074/jbc.M115.641407 [PubMed: 25873394]
55. Chen DL, Wang DS, Wu WJ, Zeng ZL, Luo HY, Qiu MZ, et al. Overexpression of paxillin induced by miR-137 suppression promotes tumor progression and metastasis in colorectal cancer. *Carcinogenesis.* 2013; 34(4):803–11. DOI: 10.1093/carcin/bgs400 [PubMed: 23275153]
56. Rychahou PG, Kang J, Gulhati P, Doan HQ, Chen LA, Xiao SY, et al. Akt2 overexpression plays a critical role in the establishment of colorectal cancer metastasis. *Proc Natl Acad Sci U S A.* 2008; 105(51):20315–20. DOI: 10.1073/pnas.0810715105 [PubMed: 19075230]
57. Bartolome RA, Garcia-Palmero I, Torres S, Lopez-Lucendo M, Balyasnikova IV, Casal JI. IL13 Receptor alpha2 Signaling Requires a Scaffold Protein, FAM120A, to Activate the FAK and PI3K Pathways in Colon Cancer Metastasis. *Cancer Res.* 2015; 75(12):2434–44. DOI: 10.1158/0008-5472.CAN-14-3650 [PubMed: 25896327]
58. Rychahou PG, Jackson LN, Silva SR, Rajaraman S, Evers BM. Targeted molecular therapy of the PI3K pathway: therapeutic significance of PI3K subunit targeting in colorectal carcinoma. *Ann Surg.* 2006; 243(6):833–42. discussion 43-4. DOI: 10.1097/01.sla.0000220040.66012.a9 [PubMed: 16772787]
59. Gulhati P, Bowen KA, Liu J, Stevens PD, Rychahou PG, Chen M, et al. mTORC1 and mTORC2 regulate EMT, motility, and metastasis of colorectal cancer via RhoA and Rac1 signaling pathways. *Cancer Res.* 2011; 71(9):3246–56. DOI: 10.1158/0008-5472.CAN-10-4058 [PubMed: 21430067]
60. Yuge R, Kitadai Y, Shinagawa K, Onoyama M, Tanaka S, Yasui W, et al. mTOR and PDGF pathway blockade inhibits liver metastasis of colorectal cancer by modulating the tumor microenvironment. *Am J Pathol.* 2015; 185(2):399–408. DOI: 10.1016/j.ajpath.2014.10.014 [PubMed: 25478811]
61. Roberts RB, Min L, Washington MK, Olsen SJ, Settle SH, Coffey RJ, et al. Importance of epidermal growth factor receptor signaling in establishment of adenomas and maintenance of carcinomas during intestinal tumorigenesis. *Proc Natl Acad Sci U S A.* 2002; 99(3):1521–6. DOI: 10.1073/pnas.032678499 [PubMed: 11818567]
62. Miyamoto Y, Suyama K, Baba H. Recent Advances in Targeting the EGFR Signaling Pathway for the Treatment of Metastatic Colorectal Cancer. *Int J Mol Sci.* 2017; 18(4)doi: 10.3390/ijms18040752
63. Tong WM, Ellinger A, Sheinin Y, Cross HS. Epidermal growth factor receptor expression in primary cultured human colorectal carcinoma cells. *Br J Cancer.* 1998; 77(11):1792–8. [PubMed: 9667648]
64. Radinsky R, Risin S, Fan D, Dong Z, Bielenberg D, Bucana CD, et al. Level and function of epidermal growth factor receptor predict the metastatic potential of human colon carcinoma cells. *Clin Cancer Res.* 1995; 1(1):19–31. [PubMed: 9815883]
65. De Jong KP, Stellema R, Karrenbeld A, Koudstaal J, Gouw AS, Sluiter WJ, et al. Clinical relevance of transforming growth factor alpha, epidermal growth factor receptor, p53, and Ki67 in colorectal liver metastases and corresponding primary tumors. *Hepatology.* 1998; 28(4):971–9. DOI: 10.1002/hep.510280411 [PubMed: 9755233]

66. Siravegna G, Mussolin B, Buscarino M, Corti G, Cassingena A, Crisafulli G, et al. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. *Nat Med*. 2015; 21(7):795–801. DOI: 10.1038/nm.3870 [PubMed: 26030179]
67. Srivatsa S, Paul MC, Cardone C, Holcman M, Amberg N, Pathria P, et al. EGFR in Tumor-Associated Myeloid Cells Promotes Development of Colorectal Cancer in Mice and Associates With Outcomes of Patients. *Gastroenterology*. 2017; 153(1):178–90 e10. DOI: 10.1053/j.gastro.2017.03.053 [PubMed: 28400195]
68. Higginbotham JN, Zhang Q, Jeppesen DK, Scott AM, Manning HC, Ochieng J, et al. Identification and characterization of EGF receptor in individual exosomes by fluorescence-activated vesicle sorting. *J Extracell Vesicles*. 2016; 5:29254.doi: 10.3402/jev.v5.29254 [PubMed: 27345057]
69. Iliou MS, da Silva-Diz V, Carmona FJ, Ramalho-Carvalho J, Heyn H, Villanueva A, et al. Impaired DICER1 function promotes stemness and metastasis in colon cancer. *Oncogene*. 2014; 33(30):4003–15. DOI: 10.1038/onc.2013.398 [PubMed: 24096488]
70. Su X, Chakravarti D, Cho MS, Liu L, Gi YJ, Lin YL, et al. TAp63 suppresses metastasis through coordinate regulation of Dicer and miRNAs. *Nature*. 2010; 467(7318):986–90. DOI: 10.1038/nature09459 [PubMed: 20962848]
71. Rokavec M, Oner MG, Li H, Jackstadt R, Jiang L, Lodygin D, et al. IL-6R/STAT3/miR-34a feedback loop promotes EMT-mediated colorectal cancer invasion and metastasis. *J Clin Invest*. 2014; 124(4):1853–67. DOI: 10.1172/JCI73531 [PubMed: 24642471]
72. Siemens H, Neumann J, Jackstadt R, Mansmann U, Horst D, Kirchner T, et al. Detection of miR-34a promoter methylation in combination with elevated expression of c-Met and beta-catenin predicts distant metastasis of colon cancer. *Clin Cancer Res*. 2013; 19(3):710–20. DOI: 10.1158/1078-0432.CCR-12-1703 [PubMed: 23243217]
73. Liu C, Kelnar K, Liu B, Chen X, Calhoun-Davis T, Li H, et al. The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. *Nat Med*. 2011; 17(2):211–5. DOI: 10.1038/nm.2284 [PubMed: 21240262]
74. Shi L, Jackstadt R, Siemens H, Li H, Kirchner T, Hermeking H. p53-induced miR-15a/16-1 and AP4 form a double-negative feedback loop to regulate epithelial-mesenchymal transition and metastasis in colorectal cancer. *Cancer Res*. 2014; 74(2):532–42. DOI: 10.1158/0008-5472.CAN-13-2203 [PubMed: 24285725]
75. Jackstadt R, Roh S, Neumann J, Jung P, Hoffmann R, Horst D, et al. AP4 is a mediator of epithelial-mesenchymal transition and metastasis in colorectal cancer. *J Exp Med*. 2013; 210(7):1331–50. DOI: 10.1084/jem.20120812 [PubMed: 23752226]
76. Keene JD. RNA regulons: coordination of post-transcriptional events. *Nat Rev Genet*. 2007; 8(7):533–43. DOI: 10.1038/nrg2111 [PubMed: 17572691]
77. King CE, Cuatrecasas M, Castells A, Sepulveda AR, Lee JS, Rustgi AK. LIN28B promotes colon cancer progression and metastasis. *Cancer Res*. 2011; 71(12):4260–8. DOI: 10.1158/0008-5472.CAN-10-4637 [PubMed: 21512136]
78. Madison BB, Jeganathan AN, Mizuno R, Winslow MM, Castells A, Cuatrecasas M, et al. Let-7 Represses Carcinogenesis and a Stem Cell Phenotype in the Intestine via Regulation of Hmga2. *PLoS Genet*. 2015; 11(8):e1005408.doi: 10.1371/journal.pgen.1005408 [PubMed: 26244988]
79. Dimitriadis E, Trangas T, Milatos S, Foukas PG, Gioulbasanis I, Courtis N, et al. Expression of oncofetal RNA-binding protein CRD-BP/IMP1 predicts clinical outcome in colon cancer. *Int J Cancer*. 2007; 121(3):486–94. DOI: 10.1002/ijc.22716 [PubMed: 17415713]
80. Vainer G, Vainer-Mosse E, Pikarsky A, Shenoy SM, Oberman F, Yeffet A, et al. A role for VICKZ proteins in the progression of colorectal carcinomas: regulating lamellipodia formation. *J Pathol*. 2008; 215(4):445–56. DOI: 10.1002/path.2376 [PubMed: 18535985]
81. Li D, Yan D, Tang H, Zhou C, Fan J, Li S, et al. IMP3 is a novel prognostic marker that correlates with colon cancer progression and pathogenesis. *Ann Surg Oncol*. 2009; 16(12):3499–506. DOI: 10.1245/s10434-009-0648-5 [PubMed: 19672661]
82. Wei Q, Huang X, Fu B, Liu J, Zhong L, Yang Q, et al. IMP3 expression in biopsy specimens of colorectal cancer predicts lymph node metastasis and TNM stage. *Int J Clin Exp Pathol*. 2015; 8(9):11024–32. [PubMed: 26617820]

83. Yoo PS, Sullivan CA, Kiang S, Gao W, Uchio EM, Chung GG, et al. Tissue microarray analysis of 560 patients with colorectal adenocarcinoma: high expression of HuR predicts poor survival. *Ann Surg Oncol.* 2009; 16(1):200–7. DOI: 10.1245/s10434-008-0209-3 [PubMed: 19009247]
84. Li N, Yousefi M, Nakauka-Ddamba A, Li F, Vandivier L, Parada K, et al. The Msi Family of RNA-Binding Proteins Function Redundantly as Intestinal Oncoproteins. *Cell Rep.* 2015; 13(11):2440–55. DOI: 10.1016/j.celrep.2015.11.022 [PubMed: 26673327]
85. Rezza A, Skah S, Roche C, Nadjar J, Samarut J, Plateroti M. The overexpression of the putative gut stem cell marker Musashi-1 induces tumorigenesis through Wnt and Notch activation. *J Cell Sci.* 2010; 123(Pt 19):3256–65. DOI: 10.1242/jcs.065284 [PubMed: 20826465]
86. Wang S, Li N, Yousefi M, Nakauka-Ddamba A, Li F, Parada K, et al. Transformation of the intestinal epithelium by the MSI2 RNA-binding protein. *Nat Commun.* 2015; 6:6517.doi: 10.1038/ncomms7517 [PubMed: 25774828]
87. Kudinov AE, Karanicolas J, Golemis EA, Boumber Y. Musashi RNA-Binding Proteins as Cancer Drivers and Novel Therapeutic Targets. *Clin Cancer Res.* 2017; 23(9):2143–53. DOI: 10.1158/1078-0432.CCR-16-2728 [PubMed: 28143872]
88. Zong Z, Zhou T, Rao L, Jiang Z, Li Y, Hou Z, et al. Musashi2 as a novel predictive biomarker for liver metastasis and poor prognosis in colorectal cancer. *Cancer Med.* 2016; 5(4):623–30. DOI: 10.1002/cam4.624 [PubMed: 26775684]
89. Li D, Peng X, Yan D, Tang H, Huang F, Yang Y, et al. Msi-1 is a predictor of survival and a novel therapeutic target in colon cancer. *Ann Surg Oncol.* 2011; 18(7):2074–83. DOI: 10.1245/s10434-011-1567-9 [PubMed: 21442350]
90. Conway AE, Van Nostrand EL, Pratt GA, Aigner S, Wilbert ML, Sundararaman B, et al. Enhanced CLIP Uncovers IMP Protein-RNA Targets in Human Pluripotent Stem Cells Important for Cell Adhesion and Survival. *Cell Rep.* 2016; 15(3):666–79. DOI: 10.1016/j.celrep.2016.03.052 [PubMed: 27068461]
91. Mongroo PS, Noubissi FK, Cuatrecasas M, Kalabis J, King CE, Johnstone CN, et al. IMP-1 displays cross-talk with K-Ras and modulates colon cancer cell survival through the novel proapoptotic protein CYFIP2. *Cancer Res.* 2011; 71(6):2172–82. DOI: 10.1158/0008-5472.CAN-10-3295 [PubMed: 21252116]
92. Vikesaa J, Hansen TV, Jonson L, Borup R, Wewer UM, Christiansen J, et al. RNA-binding IMPs promote cell adhesion and invadopodia formation. *EMBO J.* 2006; 25(7):1456–68. DOI: 10.1038/sj.emboj.7601039 [PubMed: 16541107]
93. Mahapatra L, Andruska N, Mao C, Le J, Shapiro DJ. A Novel IMP1 Inhibitor, BTYNB, Targets c-Myc and Inhibits Melanoma and Ovarian Cancer Cell Proliferation. *Transl Oncol.* 2017; 10(5): 818–27. DOI: 10.1016/j.tranon.2017.07.008 [PubMed: 28846937]
94. Kim TW, Havighurst T, Kim K, Albertini M, Xu YG, Spiegelman VS. Targeting insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) in metastatic melanoma to increase efficacy of BRAF(V600E) inhibitors. *Mol Carcinog.* 2018; doi: 10.1002/mc.22786

Figure 1. Promoters and enhancers of the metastatic cascade in colorectal cancer

Key signaling pathways are placed within the metastatic cascade to indicate the effects of signaling at different points during metastasis progression.

Table 1

Genetic mouse models of metastatic colon cancer

Model	Primary Tumor	Metastatic site(s)	Induction Method	Time point assessed (Penetrance)	Ref
<i>VilCre;Tgfbtr2^{fl};Apc^{G38N/+}</i>	adenoma & adenocarcinoma (SI)	None	Constitutive	9 & 12 months	23
<i>VilCre;Tgfbtr2^{fl};Kras^{L-SL-G12D/+}</i>	adenoma & adenocarcinoma (SI & colon)	lymph nodes & lungs	Constitutive	20 weeks (15%)	24
<i>Apc^{fl}</i>	adenoma & carcinoma (Colon)	ND	adenovirus into colon	6-35 weeks	26
<i>Kras^{L-SL-G12D/+};Apc^{fl}</i>	adenoma & carcinoma (Colon)	Liver (24 weeks)	adenovirus into colon	3+ weeks (24 wks for mets)	
<i>VilCreER;Kras^{L-SL-G12D/+}</i>	Adenoma (SI); Hyperplasia (Colon)	None	Oral-TAM	16 weeks	
<i>VilCreER;Kras^{L-SL-G12D/+};Ttp53^{fl}</i>	Adenoma (SI); Hyperplasia (Colon)	None	Oral-TAM	16 weeks	19
<i>Ink4a/Arf^{-/-};VilCreER;Kras^{L-SL-G12D/+}</i>	Proximal Colon	Lung	Oral-TAM	12 weeks (62%)	
<i>Ink4a/Arf^{-/-};VilCreER;Kras^{L-SL-G12D/+};Ttp53^{fl}</i>	Proximal Colon	Lung	Oral-TAM	12 weeks	
<i>VilCre;BRAP^{V600E/+}</i>	Hyperplasia - carcinoma (SI)	Lymph node	Constitutive	>10 months (20%)	20
<i>VilCre;LSL-BRAF^{V600E/+};Ttp53^{L-SL-R172H}</i>	More carcinomas (SI)	Lymph nodes, pancreas, lung, liver	Constitutive	10-20 months (25%)	
<i>VilCre;LSL-BRAF^{V600E/+};p16^{Ink4a}*</i>	More carcinomas (SI)	Stomach, liver, lungs	Constitutive	>10 months (25%)	
<i>VilCreER;Apc⁷¹⁶</i>	Adenoma (SI & Colon)	None*	<i>i.p.</i> TAM	5-8 weeks	*22
<i>VilCreER;Apc⁷¹⁶;Kras^{L-SL-G12D/+}</i>	Adenoma (SI & Colon)	None*	<i>i.p.</i> TAM	5-8 weeks	
<i>VilCreER;Apc⁷¹⁶;Fbxw^{7fl}</i>	Adenoma (SI & Colon)	ND	<i>i.p.</i> TAM	5-8 weeks	
<i>VilCreER;Apc⁷¹⁶;Kras^{L-SL-G12D/+};Fbxw^{7fl}</i>	Adenoma with EMT (SI & Colon)	ND	<i>i.p.</i> TAM	5-8 weeks	
<i>VilCreER;Apc⁷¹⁶;Ttp53^{L-SL-R270H}</i>	Invasive adenoma (SI & Colon)	None*	<i>i.p.</i> TAM	5-8 weeks	
<i>VilCreER;Apc⁷¹⁶;Tgfbtr2^{fl}</i>	Invasive adenoma (SI & Colon)	None*	<i>i.p.</i> TAM	5-8 weeks	
<i>VilCreER;Apc⁷¹⁶;Tgfbtr2^{fl};Ttp53^{L-SL-R270H}</i>	Invasive adenoma (SI & Colon)	None*	<i>i.p.</i> TAM	5-8 weeks	
<i>VilCreER;Apc⁷¹⁶;Tgfbtr2^{fl};Ttp53^{L-SL-R270H};Fbxw^{7fl}</i>	Invasive adenoma (SI & Colon)	Liver*	<i>i.p.</i> TAM	5-8 weeks	
<i>VilCreER;Apc⁷¹⁶;Kras^{L-SL-G12D/+};Ttp53^{L-SL-R270H}</i>	Invasive adenoma with EMT (SI & Colon)	Liver*	<i>i.p.</i> TAM	5-8 weeks	
<i>VilCreER;Apc⁷¹⁶;Kras^{L-SL-G12D/+};Tgfbtr2^{fl};Fbxw^{7fl}</i>	Invasive adenoma with EMT (SI & Colon)	Liver*	<i>i.p.</i> TAM	5-8 weeks	

Model	Primary Tumor	Metastatic site(s)	Induction Method	Time point assessed (Penetrance)	Ref
<i>VilCreER;Apc⁷¹⁶;Kras^{SL-G12D/+};Tgfbp2^{fl};Trp53^{SL-R270H}</i>	Invasive adenoma with EMT (SI & Colon)	Liver, Lung *	<i>i.p.</i> TAM	5-8 weeks	
<i>VilCreER;Apc⁷¹⁶;Kras^{SL-G12D/+};Tgfbp2^{fl};Trp53^{SL-R270H};Fbxw7^{fl}</i>	Invasive adenoma with EMT (SI & Colon)	Liver, Lung *	<i>i.p.</i> TAM	5-8 weeks	
<i>VilCreER;Apc^{fl};Trp53^{fl}</i>	Adenoma, carcinoma (Colon>SI)	None	TAM enema	1-60 weeks	
<i>VilCreER;Tet-LSL-Kras^{G12D};Apc^{fl};Trp53^{fl}</i>	Adenoma, carcinoma (Colon)	Liver (11%)	TAM enema/oral DOX	1-60 weeks post-TAM enema/oral DOX	**25
<i>VilCreER;Tet-LSL-Kras^{G12D};Apc^{fl};Trp53^{fl}</i>	Adenocarcinoma (SI & Colon)	None	TAM enema/oral DOX	1-60 weeks	
<i>VilCreER;Tet-LSL-Kras^{G12D};Apc^{fl};Trp53^{fl}</i>	Adenoma, carcinoma (Colon>SI)	Liver (25%)	TAM enema/oral DOX	1-60 weeks	

* Metastasis assessed via intrasplenic transplantation of dissociated tumor organoids into NSG and/or C57Bl/6 mice as tumors assessed 4 weeks later

NSG non-obese diabetic/severe combined immuno-deficient gamma

ND indicates that data is not included or was experiment was not performed.

Table 2

Orthotopic organoid transplantation models of metastasis

Cells of origin (organoid)	Recipient Mouse	Method of administration	Primary Tumor (penetrance)	Latency	Metastasis	Latency	Ref
Ms <i>Villin-CreERT2;Apc^{fl/f};KRAS^{G12D}/WT;p53^{R172H}</i>	NSG	Cecal wall injection	Adenocarcinoma	4-6 weeks	Liver & lungs	6-8 weeks	
Hs <i>APC^{KO};KRAS^{G12D};SMAD4^{KO}</i>	NSG	Cecal wall injection	Cystic tumors, no proliferation (67%)	16 weeks	None	ND	
Hs <i>APC^{KO};p53^{KO};SMAD4^{KO}</i>	NSG	Cecal wall injection	Relatively slow growth (67%)	16 weeks	None	ND	**10
Hs <i>KRAS^{G12D};p53^{KO};SMAD4^{KO}</i>	NSG	Cecal wall injection	Relatively slow growth (67%)	16 weeks	None	ND	
Hs <i>APC^{KO};KRAS^{G12D};p53^{KO}</i>	NSG	Cecal wall injection	Carcinoma in situ, adenocarcinoma (100%)	16 weeks	Liver & lungs (14%)	ND	
Hs <i>APC^{KO};KRAS^{G12D};p53^{KO};SMAD4^{KO}</i>	NSG	Cecal wall injection	Invasive adenocarcinoma (100%)	16 weeks	Liver & lungs (44%)	ND	
Ms <i>Apc^{mini/+};Kras^{LSL-G12D/+}; Vill1Cre;Trp53;Lgr5^{DTRαGFP}</i>	NSG	Colonic mucosal injection	Yes, type not indicated	6 weeks	None	6 weeks	**13
Ms <i>Apc^{mini/+};Kras^{LSL-G12D/+}; Vill1Cre;Trp53;Smad4;Lgr5^{DTRαGFP}</i>	NSG	Colonic mucosal injection	Yes, type not indicated	3 weeks	Liver	3 weeks	
Ms <i>LSL-rtTA/TRE-GFP-shApc</i>	Athymic-Nude-Foxn1 ^{nu}	Enema	Benign adenoma	10 days	None	Out to 1 year	
Ms <i>shApc/Kras^{G12D}</i>	Athymic-Nude-Foxn1 ^{nu}	Enema	Carcinomas	8 weeks	ND	ND	**11
Ms <i>shApc/Kras^{G12D};p53^{R172H/+}</i>	Athymic-Nude-Foxn1 ^{nu}	Enema	Carcinomas	16.5 weeks	ND	ND	
Ms <i>Apc^{mut}/Kras^{G12D}/p53^{mut}</i>	C57Bl6 (syngeneic)	Enema	Adenoma - invasive carcinoma	4-16 weeks	Liver (17%)	16 weeks	
Ms <i>Apc^{CKO};Kras^{LSL-G12D};p53^{fl/fl}</i>	C57Bl6 (syngeneic)	Colonic mucosal injection	Invasive tumor	ND	None	ND	**12
Ms <i>Apc^{CKO};Kras^{LSL-G12D};p53^{fl/fl}</i>	NSG	Colonic mucosal injection	Invasive tumor	12 weeks	Liver (33%)	12 weeks	

Ms, Mus musculus

Hs, Homo sapiens

ND indicates that data is not included or experiment was not performed.