Development of Preclinical Models to Understand and Treat Colorectal Cancer

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Abstract

The establishment and validation of preclinical models that faithfully recapitulate the pathogenesis and treatment response of human colorectal cancer (CRC) is critical to expedient therapeutic advances in the clinical management of this disease. Integral to the application of precision medicine for patients diagnosed with metastatic CRC is the need to understand the molecular determinants of response for a given therapy. Preclinical models of CRC have proven invaluable in answering many of our basic questions relating to the molecular aberrations that drive colorectal tumor progression. This review will address the comparative merits and limitations of the broad spectrum of in vitro and in vivo models available for study of colorectal tumors and their response to experimental therapies.

Keywords

- organoid
- xenograft
- genetically engineered mouse model

When clinical trials fail, poorly predictive preclinical tumor models are often blamed for injudicious advancement of cancer drug candidates. Successful drug development efforts in the colorectal cancer (CRC) arena critically depend on the availability of robust preclinical test systems that accurately model human disease. The full repertoire of preclinical cancer models needs to take into account all of the various stages of the drug discovery process, starting with early target selection and validation. Continuing through the clinical candidate selection phase and beyond, preclinical models can be a valuable resource to facilitate biomarker selection and clinical trial design. Here, we review the most commonly used in vitro and in vivo preclinical models used in CRC research today, discussing their comparative merits and limitations.

In Vitro Model Systems

Human Cell Lines

For decades, human-derived cell lines have proven to be invaluable tools for exploring the functional significance of genomic aberrations in driving tumor progression. CRC cell lines have proven useful in cancer drug discovery and

development, providing utility in target validation, biomarker identification, and assessment of anticancer drug sensitivity.^{1–3} Isogenic cell lines in particular have proven useful in the identification of therapeutic approaches and agents targeted to specific genetic alterations that are pivotal to cancer development.^{4,5} The extent to which CRC cell lines recapitulate the genetic diversity of primary cancers is an important question. Comprehensive studies have been undertaken to study as many as 150 of the most widely used CRC cell line models to address this concern.^{6,7} A detailed comparison of the genetic alterations of this CRC cell line panel to those reported for The Cancer Genome Atlas (TCGA)-analyzed primary tumors revealed close resemblance with respect to exome mutation and DNA copy-number spectra.⁶ These results further validate the use of CRC lines to perform comparative drug response assays and can be used to identify CRCs dependent on kinases for which clinically approved drugs are available.⁷

Spheroid/Organoid Models

A limitation of studies performed with two-dimensional monolayer cultures is the failure of these model systems to capture the role of the tumor microenvironment on drug

efficacy. The interplay of several factors, including hypoxia, stromal cells, and the extracellular matrix, ultimately impact the ability of drugs to reach and effect their intended targets. Engineering of an *in vitro* screening platform to accurately recapitulate the tumor microenvironment is challenging. The development of *in vitro* three-dimensional (3D) drug screening models for high passage CRC cell lines has been met with discordance between *in vitro* treatment efficacy and clinical response patterns.⁸ However, spheroids generated from CRC patient tumor samples have been found to retain the mutation profile of the parental tissue and elicit comparable tumor responses to the clinical setting.^{8,9} The establishment of 3D cultures, referred to as “organoids,” can also be derived from healthy epithelium from CRC patients to facilitate creation of a biobank of paired organoids.¹⁰ The ability of organoid cultures to capture subclonal populations makes them an ideal *ex vivo* screening platform to design personalized anticancer treatments.^{10,11} Key advantages of this platform are its amenability to rapid high throughput screening and its potential to inform the design of personalized treatment strategies more expeditiously than *in vivo* screening of xenografts established from the same specimens. However, some drugs or drug candidates, such as anti-angiogenic agents or immunotherapy, would not be expected to be active outside of an *in vivo* setting. Clinical trials are underway to validate the ultimate utility predictive value of organoid screening.

In Vivo Model Systems

Several extensive general reviews can be found elsewhere pertaining to the development of mouse models of CRC.^{12–16} Sharpless and Depinho discussed many of the features of a good mouse model, including cost considerations that dictate the need for short tumor latency and high penetrance.¹⁷ In addition, the ideal mouse model is readily evaluable, simple to use, and faithful to human disease. Xenografts and genetically engineered mouse models (GEMMs) are the two most common types of models studied in the field. We will provide an overview of each of these categories, discussing their key strengths as well as their limitations, which are summarized in ► **Table 1**. Outside the scope of this article are syngeneic models, such as mouse colon tumors that do not provoke an immune response upon transplantation, and autochthonous mouse models of CRC that originate in the colon.

High Passage Xenografts

Xenografts result from the injection of dissociated human tumor cells or surgical implantation of tumor fragments into immune-deficient mice, such as athymic nude mice, which do not produce T-lymphocytes. Xenograft-based platforms have been utilized for more than 50 years and traditionally have relied on subcutaneous injection of human tumor cell lines that have been extensively passaged *in vitro*. Roughly 1 million cultured cells are injected into each immunodeficient mouse, resulting in a palpable tumor within a few weeks of transplantation. Therefore, a cell line xenograft study repre-

sents a low cost option with a relatively rapid timeline of completion. Furthermore, CRC cell lines have been well characterized, with readily available genomic and drug response data.^{18–20} Cell lines can also easily be genetically manipulated with inducible shRNA or CRISPR/Cas9 technology, which facilitates their utility in target validation. Cell line xenografts also have utility in the early stages of drug discovery, where compound prioritization and assessment of pharmacodynamic modulation of the biochemical target are critically important.

However, with the exception of lung cancer,²¹ preclinical xenografts have provided little predictive value in directing us to tumor types that are most likely to respond to a given agent. One notable example is troglitazone, an agonist of peroxisome proliferator-activated receptor- γ , which was shown to be active against CRC, in culture and *in vivo* against cell line xenografts.²² Nevertheless, CRC patients showed rapid progression within months of initiating therapy with troglitazone.²³ There are likely many reasons for the lack of correlation of cell line xenograft and clinical outcome data, including the rigor used to interpret cell line xenograft data.^{17,24} The literature is filled with examples of agents that elicit statistically significant decreases in tumor growth rates, despite the fact that the treated tumors are still progressing. A major drawback is the failure of xenografts to capture the broad molecular diversity of CRC. Changes in genetic aberrations and the ability to maintain heterogeneous cell populations likely accompany the adaptation of cell lines to *in vitro* growth conditions, consequently impacting their reliance on specific signaling pathways.

Patient-Derived Xenografts

An emerging xenograft platform that addresses the need to preserve the histopathological and genomic characteristics of the primary tumor involves direct grafting of a fragment taken from a patient's tumor into immunodeficient mice. Patient-derived xenograft (PDX) models are more clinically relevant than cell line xenograft models based on better expression of genetic drivers characterizing the primary tumor specimens. While the study of PDX models dates back to the 1980s, interest in recent years has been renewed. The process of generating PDX models begins with procurement of tissue samples by surgery or biopsy procedures. Tumors are then implanted into immunodeficient mice as tumor fragments or single-cell suspensions. While subcutaneous implantation is the most commonly adopted method, successful orthotopic implantation of colorectal tumor material into the mouse cecum leading to liver metastasis has also been reported.^{25,26} Several laboratories have now reported on the generation of panels of CRC PDX models, which are relatively easy to establish with an engraftment rate of more than 60%.^{27–29} Importantly, there appears to be no major bias with respect to CRC subgroups, that is, localization, histologic, or molecular parameters.²⁸ The finding that the incidence of driver mutations, such as KRAS, BRAF, and PIK3CA, found in colorectal PDX models mirrors that found in the CRC patient population supports their use in studying clinically relevant disease.

Table 1 Key attributes of in vivo tumor model systems

Model system	Advantages	Drawbacks
Cell line xenografts	<ul style="list-style-type: none"> • High throughput • Amenable to study of isogenic lines • Useful to assess pharmacodynamics of drug action 	<ul style="list-style-type: none"> • Genetic drift accompanies high passage • Subcutaneous implants do not incorporate the natural tumor microenvironment • Questionable correlation with clinical outcome data
Patient-derived xenografts	<ul style="list-style-type: none"> • Tumors capture molecular heterogeneity encountered in patients • Correlation of preclinical efficacy with clinical outcome data • Facilitates study of clinically relevant drug resistance mechanisms 	<ul style="list-style-type: none"> • Requires access to surgical specimens • Labor intensive and longer timelines than cell line xenografts • Selective outgrowth of more malignant cells • Rapid replacement of human stroma by murine host
Transgenic (genetically engineered mouse models)	<ul style="list-style-type: none"> • Tumors arise in tissue of origin • Highly defined system where primary genetic defects are known • Uniquely suited for mechanistic studies 	<ul style="list-style-type: none"> • Tumors are slow to arise • Animals seldom treated as cohort and are difficult to diagnose and stage • Does not model genomic heterogeneity encountered in clinic • Low throughput and longer timelines

Even after successive passages, colorectal PDX tumors exhibit a high degree of similarity to the parental tumor with respect to histologic architecture, gene expression profile, and chromosomal instability. Roughly 10% of CRC PDX models are moderately differentiated to poorly differentiated and 10% are mucinous adenocarcinomas, which is consistent with the low prevalence of these histologic subtypes.²⁸ Very importantly, pharmacologic studies have revealed a high correlation between drug response patterns in colorectal PDX models and clinical outcome.^{28,30} Cetuximab treatment of CRC PDXs resulted in response rates similar to those observed in the clinic and were confined to the KRAS wild-type population. Neither regression nor disease stabilization was achieved in any of the KRAS-mutant CRC cohorts.³⁰ In a different study, single-agent sensitivity to irinotecan was found in nearly 40% of the CRC PDX models tested, agreeing well with the clinical response rate of roughly 20 to 30% in newly diagnosed CRC patients.^{28,31}

Their ability to faithfully capture the histologic and genomic heterogeneity observed in the clinic, coupled with concordance of the pharmacologic data, have made CRC PDX models strong translational tools. They have become a valuable resource to enable biomarker identification and the design of experimental therapeutic mouse trials to test the potential of various combination therapies to treat CRC. Examples include the finding that HER2 was amplified in a subset of cetuximab-resistant KRAS/NRAS/BRAF/PIK3CA wild-type models, just as HER2 is enriched in clinically nonresponsive KRAS wild-type patients.³⁰ This finding led Bertotti and colleagues³⁰ to carry out a proof-of-concept, multi-arm study in HER2-amplified CRC PDX models to show that combined inhibition of HER2 and EGFR was highly efficacious. Other examples include combination testing of MEK inhibitor-based regimens showing that KRAS mutant CRC PDX models regress in response to cotargeting of CDK4/6,²⁹ but not PI3K/mTOR.³²

Limitations of CRC PDX Models

Commonly cited drawbacks of PDX models (for all tissues of origin, not just CRC) include their labor- and time-intensive nature. Establishment of PDX models is highly dependent on ready access to fresh specimens, which must be implanted relatively soon after surgery or biopsy. The selective outgrowth of more malignant cells also favors tumors and clones exhibiting more aggressive cell-autonomous phenotypes.^{28,33} Finally, human tumor stroma is replaced by the murine host relatively rapidly, generally by the fourth passage. This feature of PDX models, along with the use of severely immunocompromised mice as hosts, precludes the ability to use these models to evaluate immunomodulatory or stroma-directed therapeutic agents. Despite their limitations, PDX models have clearly gained traction for their attractiveness at modeling clinically relevant disease.^{33,34} The jury is still out on their ultimate predictive value to clinical outcome. Ideally, PDX models established at the time a patient initiates standard of care treatment could eventually serve to inform the design of individualized precision medicine approaches. However, the ability of patient-derived specimens to grow in mice is not a guarantee and for many patients, the time to develop and test PDX models is too long for clinical decision making. However, the clinical impact of PDX models on precision medicine will likely be quite substantial, by elucidating biomarkers that predict sensitivity or resistance to a particular therapy.³³

Genetically Engineered Mouse Models

A critical limitation of xenograft models, whether cell line based or patient derived, is the absence of tumor-stromal interactions due to their reliance on immunosuppressed animals. In this respect, GEMMs more closely emulate human disease. A multitude of GEMMs have been developed that recapitulate genetic lesions found in sporadic or

hereditary forms of CRC. More extensive reviews on the subject of GEMMs of CRC can be found elsewhere.^{16,35,36} GEMMs have proven to be powerful tools to basic cancer researchers interested in recapitulating spontaneous tumor formation that results from critical mutations important to the initiation and progression of human cancer.

Twenty-five years ago, Fearon and Vogelstein presented evidence for a multistep genetic model of human CRC progression that begins with formation of a benign lesion in response to loss of APC function.³⁷ In this seminal study, subsequent activation of the KRAS oncogene and loss of TP53 suppressor gene function were further incorporated into their model as key events contributing to malignant disease progression. It was later discovered that multiple alternative genetic pathways to CRC exist and concomitant alterations in these three genes are found in less than 25% of human CRC.³⁸ We now know from large-scale genome-wide sequencing efforts that there exist a large number of additional genomic aberrations that coexist within the same tumor.³⁹

Apc^{Min/+} Mice: Early efforts to genetically model human CRC focused on inactivating mutations of Apc, which are found in greater than 50% of sporadic adenomas and carcinomas, and are associated with familial adenomatous polyposis (FAP). The Min (multiple intestinal neoplasias) mouse, which arose from a chemical mutagenesis screen, carries a heterozygous nonsense mutation in the Apc gene and typically develops approximately 30 polyps in the small intestine, which are generally adenomas. Apc^{Min/+} mice have been used as a platform for screening chemopreventive and therapeutic agents. However, this early GEMM is hampered by the use of germ line modification leading to an artificially high frequency of tumors developing in the small intestine distal to the duodenum. This site of disease is relatively rare in patients, with an incidence of less than 2% among CRC patients.³⁵ The vast majority of gastrointestinal tumors in FAP patients are found in the colon and rectum followed by the duodenum.

Cre-LoxP Systems: To more accurately mimic human CRC, several laboratories have relied on Cre-mediated recombination of target genes to target Apc inactivation and/or other mutations to the mouse intestinal tract. A summary of GEMMs designed in this way can be found elsewhere.³⁶ Cre-LoxP systems, which typically elicit smaller numbers of tumors in the colon, produce a greater proportion of carcinomas relative to adenomas when compared with germ line models. Notable examples include the development of a CDX2P-NLS Cre transgenic mouse, which leads to tumors demonstrating β -catenin dysregulation, biallelic loss of Apc expression, and aneuploidy.⁴⁰ This model is potentially useful for drug testing because it presents with distal colonic tumors that can be imaged. Another key example of a promising CRC GEMM model was developed by Hung and colleagues, who successfully engineered simultaneous, inducible inactivation of Apc and activation of Kras in the adult colon.⁴¹ Subsequently, this model was taken a step further by infection of colorectal mucosa with adenovirus-expressing Cre-recombinase to remove the LoxP site from a triple-mutant mouse with conditional deletion of Apc, p53, and activating Kras mutation. Although all cells in the tissue

contain LoxP elements for the targeted genes, only stem cells repopulate crypts with cells harboring malignant potential. Consequently, this technique led to a GEMM exhibiting only one or a few adenomas and carcinomas.⁴² Cell lines established from this novel GEMM were further shown to be able to develop invasive adenocarcinomas in the distal colon, providing an orthotopic CRC transplant model to carry out drug screening in a clinically relevant tumor–stroma–immune microenvironment.

Limitations of GEMMs

The success of GEMMs in increasing our understanding of cancer biology is indisputable. Their application in the drug discovery arena generally involves their usefulness in cancer target validation, as exemplified by GEMM studies showing that p53 mediates the apoptotic effects of DNA damaging agents.⁴³ However, historically these models have not played a central role in cancer drug development. Reasons for this can partially be attributed to important biological differences that exist between mouse and human cancers, including dependence on certain tumor suppressor mechanisms and differences in cytogenetic structure that can influence tumor progression.¹⁷ Furthermore, the penetrance and onset of tumor evolution in GEMMs has hindered their usefulness in drug screening, compounded by the need to select an appropriate GEMM that reliably mirrors the target in human disease.²⁴ The same attribute that renders them valuable for target validation purposes, that is, initiation and maintenance of tumors by the same genetic mutation of a cancer driver gene, is detrimental to drug screening, as the numbers of pathways to tumorigenesis are limited. The heterogeneity of human tumors results in several potential resistance mechanisms that are better addressed by PDX models.

Conclusion

Basic cancer researchers and drug discovery scientists share the mission to discover and develop improved therapies to treat CRC patients. However, there is no one perfect model platform that fulfills the collective needs of the CRC research community. An integrated strategy is warranted—one that judiciously utilizes cell-based in vitro and xenograft screens to optimize target and agent selection, followed by carefully designed PDX and GEMMs in vivo trials. All have a place and will be critical to successfully translate basic discoveries into approved drugs.

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