Mouse Models of Kras-Mutant Colorectal Cancer: Valuable GEMMs for Drug Testing?

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The development of effective therapies for colorectal cancer depends on the ability of preclinical models to faithfully recapitulate the molecular and biologic behavior of human tumors. This study reports on the characterization of colorectal genetically engineered mouse models and their derivative cell lines carrying wild-type or oncogenic Kras with concomitant Apc and p53 loss. \textit{Clin Cancer Res}; 19(11): 2794–6. ©2013 AACR.

In this issue of \textit{Clinical Cancer Research}, Martin and colleagues (1) describe the biologic properties of cell lines derived from novel genetically engineered mouse models (GEMM) of sporadic colorectal cancer carrying an oncogenic \textit{Kras} allele in the context of \textit{Apc} and \textit{p53} loss in the distal colon (Fig. 1).

More than 2 decades ago, Fearon and Vogelstein (2) proposed a model of human colorectal tumor progression in which loss of \textit{Apc} function initiates the formation of a benign lesion. This process is in turn followed by oncogenic activation of \textit{KRAS}, loss of \textit{TP53}, and of the 18q locus that altogether contribute to malignant disease progression. However, concomitant molecular alterations in \textit{APC}, \textit{KRAS}, and \textit{TP53} were later found to be present in less than a quarter of all human colorectal tumors (3, 4). Genomewide sequencing projects have further refined the molecular landscape of colorectal cancers, unveiling several dozen molecular aberrations in many additional genes that coexist within the same cancer (5).

Despite a large number of preclinical models of colorectal cancer available to researchers, none adequately captures the complexity of human disease. These include panels of human colorectal cancer cell lines that can be grown \textit{in vitro} as monolayers or in suspension as spheroids, as well as injected subcutaneously or orthotopically to form tumors in immunocompromised mice. Likewise, patient-derived xenografts have been successfully generated by implantation of fresh colorectal cancer specimens in murine hosts with severe immunodeficiency.

The critical limitations of either cell line– or patient-derived xenografts are their scarce propensity to metastasize and the requirement to use immunosuppressed mice in which complex tumor–stromal interactions may be lost.

In this respect, GEMMs closely emulate many aspects of the human disease counterpart. Several GEMMs have been developed that recapitulate genetic lesions underlying sporadic or hereditary forms of colorectal cancers. Early GEMMs were traditionally hampered by the use of germine or tissue-wide modification of frequently altered genes in colorectal cancers and by an artificially high frequency of tumors developing in the small intestine. Such site of disease is infrequent in patients, with an incidence of less than 2% of colorectal cancers.

To better mimic human disease, Hung and colleagues used a previously developed \textit{Apc} conditional knockout mouse model (6) and refined a technique to restrict tumor formation to the distal colon. This goal was achieved by adenoovirus delivery of Cre-recombinase to somatically inactivate \textit{Apc} in the local colorectal mucosa (7).

This same group has now taken the model one step further. Infection of colorectal mucosa with adenovirus-expressing Cre-recombinase is used to remove the LoxP sites from a triple-mutant mouse with conditional deletion of \textit{Apc}, \textit{p53}, and activating \textit{Kras} mutation. Although all cells in the tissue contain LoxP elements for the targeted genes, only stem cells repopulate crypts with cells that have malignant potential.

In contrast to previous colorectal cancer GEMMs that display numerous neoplastic masses in the intestine, the technique used by Martin and colleagues induces formation of only one or a few adenomas and carcinomas. Human disease progression is, therefore, closely paralleled, as very low colonic tumor burden allows for prolonged survival in mice and progression to more advanced or metastatic stages of the disease. This feature is particularly relevant for evaluating therapeutic strategies, as medical treatment is usually administered in advanced disease stages.

Despite the advantages, the GEMMs described in this study still suffer from drawbacks intrinsic to the model. The cost, the timing, and the difficulty of surgical techniques will likely hinder comprehensive drug discovery efforts. To overcome these limitations, the authors have established...
GEMM-derived cell lines. Importantly, these murine lines retain many of the biochemical and biologic features displayed by the tumors from which they are derived, making them a valuable tool for large-scale therapeutic tests. Of note, GEMM-derived cell lines are able to develop invasive adenocarcinomas in the distal colon of a syngeneic immunocompetent recipient host mouse. Murine lines are also able to form hepatic metastases when injected intrasplenically. Longer term studies will reveal whether metastases in disease-specific sites, such as the liver, thus allowing testing strategies to interfere with this process. On the other hand, translational research will also benefit from additional models, such as panels of human cell lines, xenografts, and patient-derived tumorgrafts, which may better reflect patients’ intra- and intertumor genetic heterogeneity. Only a comprehensive strategy that uses several different preclinical models will ultimately lead to improving treatment of patients with CRC.

Perhaps the most valuable feature of the colorectal cancer model described in this issue is its suitability for evaluating the efficacy of novel anticancer therapies that target the tumor microenvironment, including immunotherapies, antiangiogenic drugs, and agents directed against tumor-associated fibroblasts. This is particularly relevant within the context of colorectal malignancy, as drugs acting on the tumor milieu have already shown clinical efficacy in the metastatic setting.

The report also showed that oncogenic \( \text{Kras} \) induced biochemical activation of downstream effectors that promoted tumor proliferation in this model. \( \text{Kras} \) silencing was able to delay the growth of tumors formed by \( \text{Kras} \)-mutant murine lines, but not in wild-type counterparts. This finding indicates that \( \text{Kras} \)-mutant cells are dependent on oncogenic signaling for proliferation and represent a convenient resource for genotype-selective large-scale screenings.
The ability of the GEMMs by Martin and colleagues to predict clinical therapeutic responses has not been tested systematically. However, the authors provide data to indicate that combined treatment of a MAP-ERK kinase (MEK)—targeted agent with a dual PI3K-mTOR inhibitor could selectively affect the viability of Kras-mutant lines in vitro.

The relevance of such findings needs to be gauged in human disease, particularly as Kras-mutant colorectal tumors may be significantly more heterogeneous in terms of biologic and clinical behavior, compared with murine counterparts. Indeed, Kras-mutant colorectal cancer samples show highly variable levels of phosphorylated downstream effectors and are characterized by heterogeneous gene expression signatures (8).

It is also debatable whether, and to what extent, human Kras-mutant colorectal cancers are addicted to Kras itself. Only a small subset of mutant colorectal cancer lines are dependent upon Kras oncogenic signaling for survival (9). Preclinical studies and early clinical data have also suggested limited efficacy of drugs inhibiting Kras effectors in Kras-mutant colorectal cancers (10, 11).

These observations support the hypothesis that Kras is a key driver in the early stages of human colorectal cancer tumorigenesis. However, it may become dispensable in later stages when tumors acquire additional genetic variants that can activate alternative or redundant signaling pathways.

GEMMs and their derivative cell lines are engineered to carry well-defined and limited numbers of genetic elements. The specific nature of these aberrations may further limit the model’s application, as it is known that individual variants in TP53 or Kras can exert different functional impact on human colorectal tumors. It is still possible that the concomitant loss of p53 and Apc might contribute to genomic instability in murine tumors, thereby promoting the spontaneous acquisition of additional genetic aberrations. Even so, GEMMs are unlikely to recapitulate the high degree of intra- and intertumoral heterogeneity observed in human colorectal cancers. In this respect, patient-derived xenograft models may better phenocopy the genetic heterogeneity observed in human colorectal cancer samples. Nevertheless, their ability to reliably predict human therapeutic response and clinical outcomes is limited to drugs acting on the tumor cell compartment.

In conclusion, an integrated strategy involving GEMMs and patient xenograft models, as well as colorectal cancer cell lines, in drug testing experiments will be required to successfully translate effective therapeutic strategies to the clinic.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: F. Di Nicolantonio, A. Bardelli
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