Mouse models of Kras mutant colorectal cancer: valuable GEMMs for drug testing?

Mouse models of *Kras* mutant colorectal cancer: valuable GEMMs for drug testing?

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Abstract

The development of effective therapies for colorectal cancer depends on the ability of preclinical models to faithfully recapitulate the molecular and biological behavior of human tumors. This study reports the characterization of colorectal GEMMs and their derivative cell lines carrying wild-type or oncogenic Kras with concomitant Apc and p53 loss.

In this issue of *Clinical Cancer Research*, Martin and colleagues describe the biological properties of cell lines derived from novel genetically engineered mouse models (GEMMs) of sporadic colorectal cancer (CRC) carrying an oncogenic *Kras* allele in the context of *Apc* and *p53* loss in the distal colon (1) (Figure 1).

Over two decades ago Fearon and Vogelstein proposed a model of human colorectal tumor progression (2) in which loss of *APC* function initiates the formation of a benign lesion. This is in turn followed by oncogenic activation of *KRAS*, loss of *TP53* and the 18q locus that altogether contributes to malignant disease progression. However, concomitant molecular alterations in *APC*, *KRAS* and *TP53* were later found to be present in less than a quarter of all human CRC tumors (3, 4). Genome-wide sequencing projects have further refined the molecular landscape of CRC unveiling several dozen molecular aberrations in many additional genes that co-exist within the same cancer (5).

Despite a large number of preclinical models of CRC available to researchers, none adequately captures the complexity of human disease. These include panels of human CRC cell lines that can be grown 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 CRC 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, genetically engineered mouse models (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 CRC. Early GEMMs were traditionally hampered by the use of germline or tissue-wide modification of frequently altered genes in CRC and by an artificially high frequency of tumors developing in the small intestine. Such site of disease is infrequent in patients, with incidence less than 2% of CRC.

To better mimic human disease, Hung and colleagues employed a previously developed *Apc* conditional knock-out mouse model (6) and refined a technique to

restrict tumor formation to the distal colon. This was achieved by adenovirus delivery of Cre-recombinase to somatically inactivate *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 employed to remove the LoxP sites from a triple mutant mouse with conditional deletion of *Apc*, *p53* and activating *Kras* mutation. While 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 CRC GEMMs that display numerous neoplastic masses in the intestine, the technique used by Martin et al. induces formation of only one or few adenomas and carcinomas. Human disease progression is, therefore, closely paralleled as very low colonic tumor burden allows for prolonged mice survival and progression to more advanced or metastatic stages of the disease. This 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 biological 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 liver, peritoneum or lung can arise spontaneously in these models. It will be equally pertinent to assess how these GEMMs and cell lines are modified by additional genetic variants commonly found in CRC, such as alterations in the PI3K/PTEN or TGF-beta signaling pathways. It is plausible that additional mutations might also trigger an early metastatic switch.

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

The report also demonstrated that oncogenic *Kras* induced biochemical activation of downstream effectors that promoted tumor proliferation in this model. *Kras* silencing was able to delay the growth of tumors formed by *Kras* mutant murine lines but not in

wild-type counterparts. This indicates that *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 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 CRC tumors may be significantly more heterogeneous in terms of biological and clinical behavior compared to murine counterparts. Indeed, *KRAS* mutant CRC 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 CRCs are addicted to *KRAS* itself. Only a small subset of mutant CRC 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 CRC (10, 11).

These observations support the hypothesis that *KRAS* is a key driver in the early stages of human CRC 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, since 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, thereby promoting the spontaneous acquisition of additional genetic aberrations. Even so, GEMMs are unlikely to recapitulate the high degree of intratumoral and intertumoral heterogeneity observed in human CRC cancers. In this respect, patient-derived xenograft models may better phenocopy the genetic heterogeneity observed in human CRC 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 CRC cell lines, in drug testing experiments will be required to successfully translate effective therapeutic strategies to the clinic.

Legend to Figure 1.

Useful mouse models of CRC for translational research need to replicate human disease as faithfully as possible. The GEMMs and derivative murine lines described by Martin and colleagues display many desirable features and open exciting perspectives. GEMM-derived lines will allow future large-scale drug screening on cells carrying well-defined genetic alterations which occur frequently in human sporadic CRC. The ability of these models to form invasive carcinomas in immunocompetent hosts will offer the opportunity to assess therapeutic strategies targeting cancer-stroma interactions. Future studies will investigate the propensity of GEMM tumors and lines to metastasize to relevant 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 inter-tumor genetic heterogeneity. Only a comprehensive strategy which employs several different preclinical models will ultimately lead to improving treatment of CRC patients.

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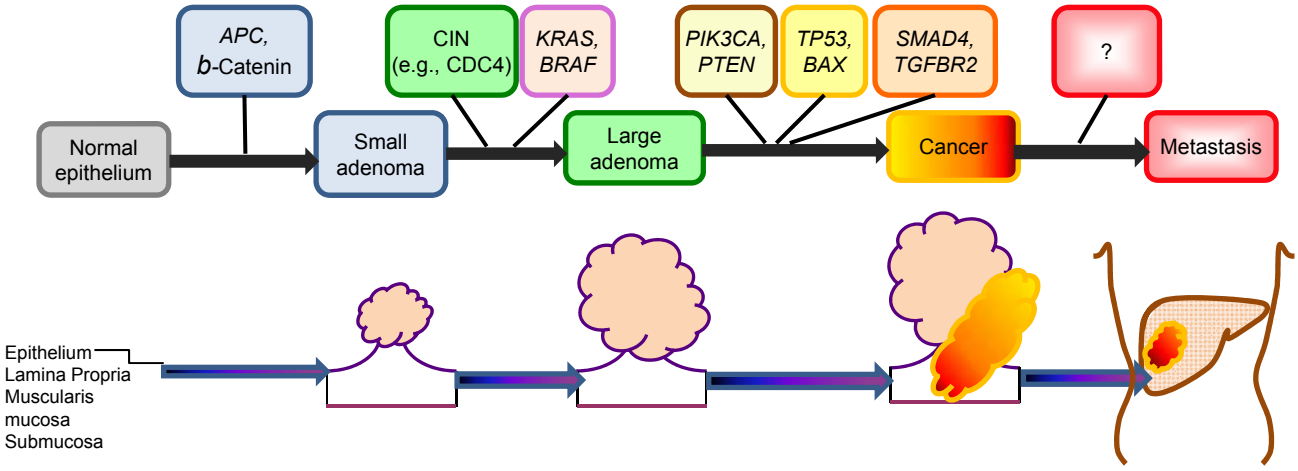
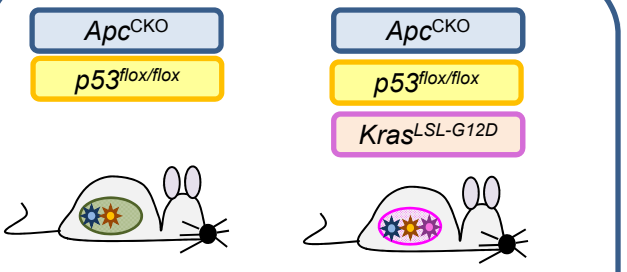
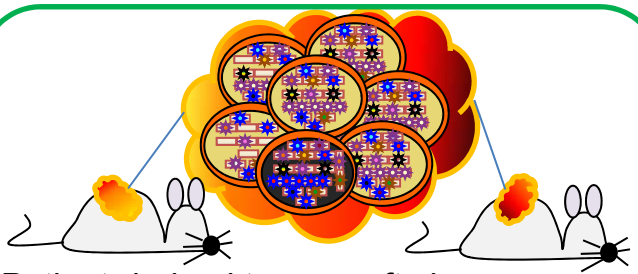
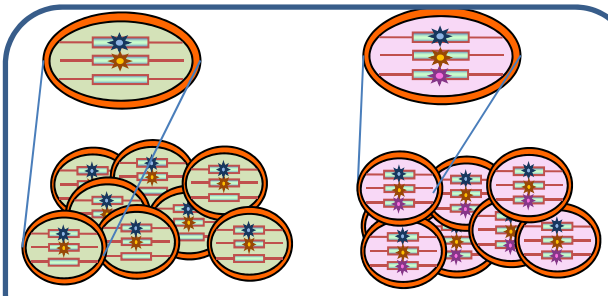




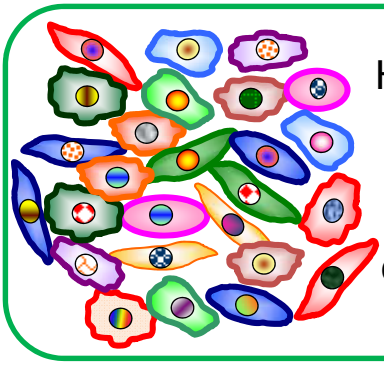
GEMMs – Distal Colon Tumors



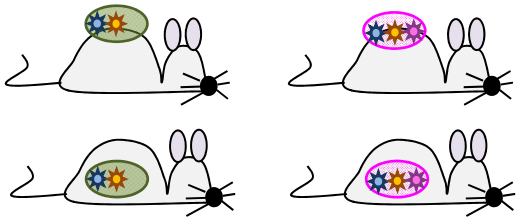
Patient-derived tumorgrafts in immunodeficient mice (often ectopic site)



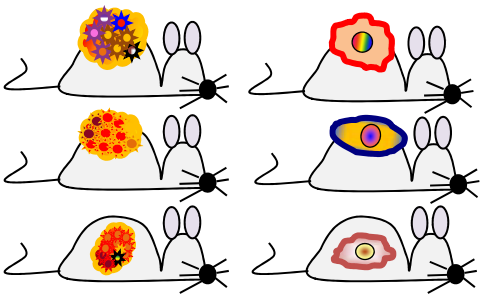
GEMM-derived cell lines



Human CRC cell lines: directly from patients or tumorgrafts, continuous lines



Ectopic and orthotopic tumors from GEMM-derived cell lines in immunocompetent mice (liver metastases?)



Ectopic and orthotopic tumors from cell lines and patient tumorgrafts

- Studies of carcinogenesis and cancer prevention
- Elucidate mechanisms of metastasis development
- Test therapies to prevent metastasis formation and to treat established metastases
- Study mechanisms of primary and acquired resistance to anticancer drugs