Development of a Colon Cancer GEMM-Derived Orthotopic Transplant Model for Drug Discovery and Validation


Abstract

Purpose: Effective therapies for KRAS-mutant colorectal cancer (CRC) are a critical unmet clinical need. Previously, we described genetically engineered mouse models (GEMM) for sporadic Kras-mutant and non-mutant CRC suitable for preclinical evaluation of experimental therapeutics. To accelerate drug discovery and validation, we sought to derive low-passage cell lines from GEMM Kras-mutant and wild-type tumors for in vitro screening and transplantation into the native colonic environment of immunocompetent mice for in vivo validation.

Experimental Design: Cell lines were derived from Kras-mutant and non-mutant GEMM tumors under defined media conditions. Growth kinetics, phosphoproteomes, transcriptomes, drug sensitivity, and metabolism were examined. Cell lines were implanted in mice and monitored for in vivo tumor analysis.

Results: Kras-mutant cell lines displayed increased proliferation, mitogen-activated protein kinase signaling, and phosphoinositide-3 kinase signaling. Microarray analysis identified significant overlap with human CRC-related gene signatures, including KRAS-mutant and metastatic CRC. Further analyses revealed enrichment for numerous disease-relevant biologic pathways, including glucose metabolism. Functional assessment in vitro and in vivo validated this finding and highlighted the dependence of Kras-mutant CRC on oncogenic signaling and on aerobic glycolysis.

Conclusions: We have successfully characterized a novel GEMM-derived orthotopic transplant model of human KRAS-mutant CRC. This approach combines in vitro screening capability using low-passage cell lines that recapitulate human CRC and potential for rapid in vivo validation using cell line-derived tumors that develop in the colonic microenvironment of immunocompetent animals. Taken together, this platform is a clear advancement in preclinical CRC models for comprehensive drug discovery and validation efforts. Clin Cancer Res; 19(11); 2929–40. ©2013 AACR.

Introduction

Activating KRAS mutations are observed in 40% to 50% of human colorectal cancer (CRC) and pose a significant therapeutic challenge because of their inherent resistance to anti-EGF receptor (EGFR) antibodies, such as cetuximab (Erbitux) or panitumumab (Vectibix; ref. 1). Although this underscores the urgent need for development of novel therapeutic strategies, the overall success rate for the clinical approval of oncology drugs continues to be less than 10% (2). As the largest failure rates occur when efficacy in human patients is first directly assessed (phase II trials), robust preclinical models that faithfully model human disease are critical to maximize the efficiency of the clinical drug development pipeline.

The majority of CRC genetically engineered mouse models (GEMM) use germline or tissue-wide modification of genes that are critical for CRC carcinogenesis (3). Although these are useful models for hereditary cancer predisposition syndromes, such as familial adenomatous polyposis and Lynch syndrome, they are poor surrogates for sporadic CRC,
which comprise approximately 80% of all CRC cases (4). Furthermore, the majority of these murine tumors present in the small intestine rather than the colon. To circumvent this problem, we have recently described novel GEMMs for sporadic CRC based on the delivery of adenovirus expressing Cre recombinase (AdCre) in a restricted fashion to the distal colon of floxed mice (5). This is a faithful surrogate for human sporadic CRC, as it is based on stochastic and somatic modification of genes known to be important in human CRC, resulting in colonic tumors that develop in the context of the colonic microenvironment of immunocompetent mice. We have successfully used this model to stratify multiple therapeutic responses according to the underlying tumor genotype (5, 6). Although this is a powerful approach that recreates human CRC with the utmost fidelity, it is more suitable for hypothesis-driven mechanistic interrogation of specific targeted therapies, rather than large-scale high-throughput drug discovery efforts.

To create a high-throughput drug discovery validation platform that closely mimics human CRC, we developed a novel GEMM-derived orthotopic transplant model that combines the capability for traditional in vitro high-throughput drug screening with rapid in vivo validation in the context of a species-matched tumor–stroma microenvironment and an intact immune system. Several high-throughput drug screening approaches rely on the use of preexisting highly passaged human CRC cell lines with poorly defined genetics; in addition, investigators have used patient-derived tumor graft models in which a human tumor fragment is serially passaged in an immunodeficient mouse host to study its biologic characteristics and response to therapeutics. Here, we have used primary tumor tissue from our GEMMs for sporadic CRC to derive low passage, genetically defined cell lines, thus providing a platform for rapid in vitro drug discovery. Furthermore, to facilitate rapid in vivo candidate drug validation, we developed a procedure to engraft these cell lines into the native colonic environment of immunocompetent mice, thus modeling the appropriate tumor-stroma-immune microenvironment for CRC carcinogenesis.

Thus, GEMMs have become a useful system in which to recapitulate and investigate a broad spectrum of human diseases, including cancers that derive from somatic genetic events. As is the case with any model of human disease condition, there are benefits as well as limitations to GEMMs as models of human carcinogenesis. Our CRC GEMMs have clear advantages over traditional human tumor cell lines. Namely, they are low passage, genetically defined, and can be studied within the context of an immune-competent colonic microenvironment. Indeed, there are limitations with any GEMMs of human disease, including the possibility that there may be aspects of human CRC not fully captured within the context of the limited driver mutations we have engineered into these models. In addition, the extent to which these models recapitulate the therapeutic response seen in human patients with CRC may also be incomplete. Nonetheless, we feel that a successful preclinical effort must take advantage of all possible models of the human disease condition, and that these GEMMs play a useful role within such a comprehensive effort.

Since KRAS mutational status is an independent predictor of treatment response in CRC (1), we generated GEMMs of advanced CRC based on: (i) somatic loss of the tumor suppressors Apc and p53 (AP) and (ii) Apc and p53 loss with concomitant gain of an activating Kras mutation (AKP). Subsequently, we derived corresponding panels of murine cell lines and showed that they contain many canonical features of human CRC biology. Finally, we successfully engrafted these cell lines into the appropriate colonic microenvironment of immunocompetent mice and observed robust tumor growth and development. Here, we present the development, characterization, and validation of our novel discovery validation platform and highlight its use by showing: (i) the predisposition of Kras-mutant tumors and tumor-derived cell lines toward enhanced growth and proliferation; (ii) the dependency of Kras-mutant tumors on oncogenic signaling; (iii) the generation of a Kras signature which closely resembles several recently published CRC signatures and is enriched for hallmark CRC characteristics; (iv) the propensity of Kras-mutant tumors towards aerobic metabolism; and (v) how this phenotypic difference might be exploited for positron emission tomography (PET)-based diagnoses of Kras-mutant tumors.
generate compound mutant mice, \( Apc^{CKO} \) and \( p53^{flox/flox} \) mice were crossed to generate \( Apc^{CKO}p53^{flox/flox} \) (AP) mice and \( Apc^{CKO}, Kras^{LSL-G12D} \), and \( p53^{flox/flox} \) mice were crossed to generate \( Apc^{CKO}, Kras^{LSL-G12D}, \) and \( p53^{flox/flox} \) (AKP) mice.

**Adenoviral infection of the colonic epithelium and endoscopy**

Twelve-week-old mice were infected with \( 10^9 \) pfu Ad5CMVcre (AdCre; Gene Transfer Vector Core, University of Iowa, Iowa City, IA) after surgical laparotomy under anesthesia (2% isoflurane), as described (5). Resulting compound genotypes and nomenclature are depicted in Supplementary Fig. S1. Mice were followed with biweekly optical colonoscopy for tumor development. The tumor size index (TSI) based on the relative ratio of the cross-sectional areas of the tumor to that of the colonic lumen was derived as described (5, 6). Tumor-bearing mice with TSI more than 75 were euthanized, and the colons were removed, opened longitudinally, and rinsed with cold PBS. Tumors fragments were isolated for genomic analysis, histology, and cell line establishment. This research protocol was approved by our attending veterinarian, by the Tufts Medical Center Institutional Animal Care and Use Committee (IACUC), and by the Tufts Medical Institutional Biosafety Committee (Boston, MA).

**Derivation of tumor cell lines**

Tumor samples were washed in 0.04% sodium hypo-chlorite followed by multiple PBS–gentamycin washes. Samples were minced and digested in collagenase D/dis-pase (Roche), collected by centrifugation, and digested further in 0.3% pancreatin (Roche) at 37°C. Digested samples were passed through a 40 μm cell strainer (BD Biosciences), collected by centrifugation, resuspended in defined culture media (Dulbecco’s modified Eagle’s medium, 10% FBS, 0.2 ng/mL EGF, 1× insulin-transferrin-selenium (Invitrogen), 2.5 μg/mL gentamycin), and plated on 60 mm² collagen IV-coated dishes (BD Biosciences). Mutational analysis was confirmed using our genotyping protocol as described in the Supplementary Methods.

Additional detailed methods are available in the Supplementary Methods.

**Results**

**Kras^{G12D} accelerates carcinogenesis in GEMM models of sporadic CRC**

Colonic tumors were induced through surgical administration of AdCre to generate \( Apc^{CKO}p53^{flox/flox} \) (AP) and \( Apc^{CKO}, Kras^{LSL-G12D}p53^{flox/flox} \) (AKP) tumors, respectively (Supplementary Fig. S1), and followed by optical colonoscopy (Fig. 1A). Although initial tumor formation was observed in AP mice

![Figure 1](https://www.aacrjournals.org/figures/2013/02/12/clincancerres.12-2307-01-acl.pdf)
by 7 weeks after surgical induction, neoplastic lesions were detected in AKP mice in as little as 2 weeks. Analysis of composite tumor growth curves further confirmed more robust tumor growth in AKP compared with AP mice (Fig. 1B). In addition, the rate of histologic progression was accelerated in AKP mice (Fig. 1C and D). Collectively, these data suggest that somatically activated oncogenic \( \text{Kras}^{G12D} \) promotes tumor progression in the context of an \( \text{Apc}^{CKO} \text{p53}^{-/-} \) genetically permissive background.

\( \text{Kras}^{G12D} \) increases MAPK and PI3K/mTOR signaling but has no effect on WNT signaling in colonic tumors

We have previously shown that introduction of \( \text{Kras}^{G12D} \) activates the downstream mitogen-activated protein kinase (MAPK) pathway in tumors derived from \( \text{Apc} \) mutation (5). To examine whether a similar response exists in tumors harboring an additional \( \text{p53} \) mutation, protein analysis was conducted by Western blot to compare levels of p-MEK and p-ERK in individual AP and AKP tumors. While levels of total MEK and ERK were equivalent, AKP tumors showed elevated levels of p-MEK and p-ERK throughout their progression (Fig. 2A). These findings, along with activation of phosphoinositide-3 kinase (PI3K) and mTOR signaling, were confirmed by immunohistochemistry (Fig. 2B). Next, we examined nuclear and cytoplasmic levels of \( \beta \)-catenin and found that tumors from both AP and AKP mice showed specific accumulation relative to adjacent normal mucosa, which is indicative of aberrant WNT signaling (Supplementary Fig. S2B). Taken together, these data suggest that \( \text{Kras}^{G12D} \) activates MAPK and PI3K/mTOR signaling and accelerates tumor progression in the context of colonic tumors with \( \text{Apc} \) and \( \text{p53} \) deficiency.

\( \text{AP} \) and \( \text{AKP} \) tumor-derived cell lines are robust in vitro models for CRC

To interrogate \( \text{Kras}^{G12D} \) CRC biology in the context of \( \text{Apc} \) and \( \text{p53} \) deficiency, we derived low-passage primary cell lines from AKP and AP tumors. These cell lines were robust models for colorectal cancer and allowed us to study the effects of \( \text{Kras}^{G12D} \) on cellular proliferation, survival, and invasion in vitro.
lines from individual AP and AKP colonic tumors. To verify proper recombination of the genetically engineered alleles, we used PCR to examine both primary tumors and cell lines. As expected, both AP and AKP primary tumors, which are composed of a heterogeneous mixture of tumor cells, supporting stroma, and immune cells, contain a mixture of both nonrecombined and recombinant alleles. After short-term passage (P<3) in vitro, PCR analysis showed uniform loss of the nonrecombined alleles (Fig. 3A). This suggests that the epithelial cells harboring genetically modified alleles obtain a growth advantage in vitro and that these cells eventually dominate the cultured cell population.

Next, we examined the in vitro and in vivo growth characteristics of AP and AKP cell lines. Cumulative population doublings (CPD) show that AKP cells grow at a faster rate compared with AP cells (~0.5 vs. 1.5 PDs per day, Fig. 3B). To further characterize in vitro growth capabilities, AP and AKP cell lines were grown in spheroid-inducing conditions. Consistent with their ability to grow more rapidly as a monolayer, AKP lines also form larger spheroids as evidenced by microscopy as well as a viability assay (Fig. 3C). Finally, we conducted subcutaneous injections of AP and AKP cells into nude mice. As indicated in Fig. 3D, AKP lines displayed an enhanced ability to grow in an immunocompromised in vivo setting compared with AP lines. Taken together, these data show that the addition of Kras in vivo confers a growth advantage in vitro and in vivo.

Mutant Kras tumor-derived cell lines are dependent on oncogenic signaling

Because of elevated MAPK and PI3K/AKT signaling in vivo (Fig. 2), we hypothesized that Kras-mutant tumor-derived cell lines would also be highly dependent on oncogenic signaling. First, Kras dependency was assessed in AP and AKP cell lines, by examining the effects of 2 independent hairpins targeting Kras. As shown in Supplementary Fig. S5A, AKP cell lines exhibited a marked decrease in cell viability upon Kras knockdown using small interfering RNA, whereas AP cell lines were not significantly affected despite protein knockdown being comparable (Supplementary Fig. S5B). These results indicated that the AKP cell lines tested displayed a greater dependency on Kras than AP cell lines, which is consistent with our hypothesis of Kras-mutant cell lines require oncogenic Kras for survival. To interrogate the degree of Kras dependency in AKP cell lines in vivo, we conducted inducible RNA interference of Kras in established tumors in vivo. Following doxycycline administration, pronounced growth inhibition was observed relative to noninduced control tumors (Fig. 4A). Following 4 days of doxycycline treatment, we examined tumors from both treated (n = 3) and untreated (n = 3) mice by Western blot analysis. Tumors exposed to doxycycline showed robust knockdown of Kras and consequent loss of p-ERK, suggesting that AKP tumor growth in vivo is reliant on MAPK signaling endowed by oncogenic Kras (Fig. 4B).
Next, we examined the effect of MAPK and PI3K/mTOR inhibition using the pathway selective small-molecule inhibitors PD-0325901 and PF-04691502, respectively. Treatment with the MEK inhibitor PD-0325901 revealed significantly greater sensitivity ($P < 10^{-7}$, Fig. 4C) in Kras-mutant cells, whereas treatment with the PI3K/mTOR inhibitor PF-04691502 showed a slightly enhanced sensitivity in Kras-mutant cells to a lower dose ($50$ nmol/L, $P < 0.01$; Fig. 4C). Subsequently, a 3-fold increase in PF-04691502 concentration had a greater impact on cell viability with AKP cells exhibiting greater sensitivity to drug treatment ($P < 10^{-5}$, Fig. 4C). Treatment using combinations of each drug showed an additive effect in both AKP and AP cells with AKP cells showing a marked decrease in viability when compared with non-Kras–mutant AP cell lines ($P < 10^{-6}$, Fig. 4C). As expected, Western blot analysis of cells exposed to individual doses of PD-0325901 and PF-04691502 showed a robust attenuation of p-MAPK and p-PI3K/AKT signaling, respectively, at 6 hours (not shown) and 24 hours (Fig. 4D). A decrease in both effector pathway phosphosignaling was observed in both AKP and AP cells with combinations of PD-0325901 and PF-04691502 after 6 hours of treatment (not shown); however, restoration of both p-AKT$^{Thr308}$ and p-AKT$^{Ser473}$ occurred only in non-Kras–mutant AP cells after 24 hours (Fig. 4D). In addition, the appearance of the apoptotic marker cleaved PARP arose only in AKP cells following a 24-hour treatment with either PD-0325901 or the combination of PD-0325901 and PF-04691502 (Fig. 4D) and is consistent with the decreased viability observed (Fig. 4C). These results suggest that an acquired or intrinsic non-Kras$^{G12D}$–mediated bypass mechanism confers relative resistance in AP cells through the induction of p-AKT. Collectively, these data show that Kras-mutant cell line growth and viability is dependent on oncogenic signaling, as well as Kras itself.

Transcriptomic analysis reveals tumor-derived cell lines capture relevant signatures of human CRC

To further characterize the extent to which our GEMM models recapitulate human disease, gene expression profiling was conducted on cell lines derived from 13 murine colon adenocarcinomas (analysis scheme outlined in Fig. 5A). The 500 most significantly varied genes were identified using a 2-sample $t$ test (FDR < 0.05, 2-fold change) and used
for hierarchical unsupervised clustering of 3 AP and 10 AKP cell lines. As indicated in Fig. 5B, cell lines clustered into 2 main groups and in concordance with their respective Kras genotypes. Differences in gene expression between AKP vs. AP cell lines was further refined by conducting supervised clustering using a composite of published Kras gene signatures (Kras Sig, see Supplementary Methods). This approach was also able to discriminate successfully between AKP and AP status (Fig. 5C).

To determine whether differential gene expression among AKP and AP cell lines contains any significant overlap with published comparative murine human signature gene datasets, pairwise comparisons were made to several CRC-related and KRAS signatures (7–9). Briefly, the genes differentially expressed in AKP versus AP were mapped to human orthologues (renamed AKP vs. AP signature, see Supplementary Table S1) and compared with humanized gene signatures of interest, including a composite KRAS signature (9), a CRC metastasis signature (10), and intestinal epithelial signatures (ISC; ref. 11). As indicated in Fig. 5D, our list of differentially expressed genes displays significant overlap with several published CRC datasets. Notably, the AKP versus AP gene set shows a striking overlap with a composite KRAS signature dataset (Kras Sig, Fig. 5C) In addition, a significant overlap was observed between our signature with 2 ISC signatures (Lgr5hi and Ephb2hi), a late TA signature, and a CRC signature derived from a murine experimental metastasis model (11).

To determine whether our AKP versus AP signature was enriched in known disease gene sets or pathways, we used Ingenuity Pathway Analysis software. The top 5 biologic disease categories are listed in Supplementary Table S2.

Figure 5. Gene expression analysis of AP and AKP cell lines. A, workflow of genomic analysis conducted on AP and AKP cell lines. B, unsupervised clustering results based on the 500 most varied genes from 3 AP and 10 AKP cell lines. Log₂-transformed data were further adjusted by means and then clustered. Cell lines clearly cluster based on genotype. C, supervised clustering was conducted on the 3 AP and 10 AKP cell lines, using an independent Kras signature gene list (Kras Sig, see Materials and Methods). A total of 544 differentially expressed genes were used in this clustering (see Materials and Methods). Log₂-transformed data was adjusted and clustered as in (B). Again, cell lines cluster based on genotype. D, pairwise comparisons using the AKP versus AP signature and published CRC signatures. A detailed description of each signature is provided in the Supplementary Methods.
Upon tumor formation, 18F-fluorodeoxyglucose (FDG) was administered via tail vein injection and mice were imaged by PET. As anticipated, FDG-PET produced an appreciable signal in AP compared with AP cell lines (Fig. 6A), with OCR remaining relatively similar in the 2 groups (Supplementary Fig. S3). In addition, AKP cell lines displayed enhanced glucose uptake compared with AP lines (Fig. 6B). Taken together, these data indicate that activating Kras mutation may affect the metabolic characteristics of these cell lines.

Given this differential metabolic profile in vitro, we wished to confirm this finding in vivo. To this end, we prepared subcutaneous tumors from AP and AP-K mutant cell lines. Upon tumor formation, 18F-fluorodeoxyglucose (FDG) was administered via tail vein injection and mice were imaged by PET. As anticipated, FDG-PET produced an appreciable signal in heart (H), spine (S), and tumor (T). As indicated in Fig. 6C, subcutaneous tumors from Kras-mutant AKP lines produced a significantly higher FDG-PET signal. In addition, both mean and maximum calculated standardized uptake values (SUV) were greater in AKP lines (Supplementary Fig. S3C and S3D). To determine whether oncogenic Kras contributed to a concomitant increase in lactate production, intratumor lactate concentration was measured biochemically. Subcutaneous tumors from Kras-mutant AKP cell lines had significantly more lactate than those with wild-type Kras (Fig. 6D), a finding consistent with the Warburg effect. Taken together, these data indicate that oncogenic Kras contributes to enhanced glucose uptake and glycolytic metabolism in our model system. In addition, given these findings, we conducted a further investigation of our transcriptomic data to assess whether glucose transporters were differentially regulated. Indeed, the glucose transporter SLC2A1 (GLUT-1) is differentially expressed in our Kras-mutant models, as indicated in Supplementary Fig. S6, further suggesting that alterations in Kras may lead to dysregulation of relevant metabolic programs.

Discussion

Activating KRAS mutations are commonly seen in 40% to 50% of human CRC (13). Although the prognostic role of such mutations is controversial, it is evident that they are powerful positive predictors for resistance to treatment with anti-EGFR therapies, such as cetuximab or panitumumab (14, 15). Because of the central role for KRAS in EGFR and other receptor tyrosine kinase signaling pathways during CRC carcinogenesis (16), robust KRAS-specific treatments are desperately needed for effective CRC treatment. As such efforts have been widely unsuccessful to date (17), the development of novel therapeutic approaches for treatment of KRAS-mutant CRC is a critical unmet clinical need.

Despite the identification of many candidate drug compounds during preclinical discovery efforts, the subsequent rate of success through the clinical trial pipeline is abysmal (18). Traditional preclinical drug discovery platforms often rely on genetically undefined and highly passaged human tumor cell lines. The in vitro discovery phase is often followed by in vivo validation in immunodeficient mice that suffer from complete species mismatch in the tumor microenvironment. This is a significant shortcoming in light of determining whether our GEMM tumor cell lines could recapitulate metastatic growth. To this end, we conducted intrasplenic injections of 2 independent AKP cell lines and observed growth in the liver. As indicated in Supplementary Fig. S4, both lines created appreciable tumor masses in the liver, as indicated by gross pathology, hematoxylin and eosin (H&E) staining and PET/computed tomography (Supplementary Fig. S4A, S4B, and S4C, respectively). Thus, these results show that our GEMM tumor cell line models can be used to test future preclinical therapeutic strategies for treating metastatic lesions.

Orthotopic transplantation of cell lines into the distal colon results in invasive adenocarcinoma

To create an orthotopic model of invasive adenocarcinoma in an immunocompetent host, we established a method for transplanting cell lines derived from primary colorectal tumors into the native colonic environment of a syngeneic immunocompetent recipient host mouse (see Supplementary Methods). A representative endoscopic image of a tumor established from an injection of AKP cells is shown in Supplementary Fig. S2A. Resulting tumors were excised after 5 weeks and sectioned. As indicated in Supplementary Fig. S2B, H&E sections of tumor and adjacent normal tissue indicated that these tumors are poorly differentiated, whereas immunohistochemical analysis found that tumor regions contained elevated nuclear β-catenin and phosphorylated constituents of the MAPK pathway compared with adjacent normal tissue, markers of deregulated WNT signaling, and colonic transformation. Furthermore, staining for Ki67 and PECAM (CD31), markers of mitosis and angiogenesis, respectively, indicated an elevated proliferation rate and angiogenic response within tumor areas, as compared with adjacent normal colon tissue (Supplementary Fig. S2B).

Intrasplenic injection of cell lines results in tumor formation at an orthotopic metastatic site

Given that the common therapeutic approach to treating advanced CRC is to treat the metastatic sites, we sought to
determine whether our GEMM tumor cell lines could recapitulate metastatic growth. To this end, we conducted intrasplenic injections of 2 independent AKP cell lines and observed growth in the liver. As indicated in Supplementary Fig. S4, both lines created appreciable tumor masses in the liver, as indicated by gross pathology, hematoxylin and eosin (H&E) staining and PET/computed tomography (Supplementary Fig. S4A, S4B, and S4C, respectively). Thus, these results show that our GEMM tumor cell line models can be used to test future preclinical therapeutic strategies for treating metastatic lesions.
the increasing evidence implicating the critical interplay between the tumor, its supporting stroma, and the surrounding immunologic milieu (19). As the highest failure rate occurs during phase II testing when clinical efficacy in humans is first directly tested, preclinical models that are better surrogates for human disease are needed (20). GEMMs possessing conditional alleles that recapitulate mutations known to be important in human disease provide an attractive and powerful alternative, as they recapitulate the full histologic, genomic, transcriptomic, and proteomic spectrum of disease seen in human disease (21, 22). Indeed, numerous successful cancer discovery and validation efforts have been possible through the use of comprehensive comparative oncogenic and oncoproteomic analyses (22–26). Furthermore, such GEMMs can be used to derive highly successful predictions of eventual therapeutic response in human disease (5, 27). Although GEMMs are excellent surrogates for human disease, they are more suited for specific hypothesis-driven efforts rather than high-throughput screening. To address this deficiency, we wished to develop a composite platform composed of (i) high-throughput in vitro screening based on CRC GEMMs and (ii) closely linked in vivo validation that faithfully recapitulates human disease.

Many CRC GEMMs use germline or tissue-wide modification of genes involved in human carcinogenesis that
paradoxically results in a high multiplicity of predominantly small intestinal tumors (28). Although GEMM-derived cell lines have been described for models of lung and pancreatic cancer (29), CRC GEMM-derived cell lines have not. This may because the large tumor burden in most CRC GEMMs results in premature death, thereby precluding the isolation and characterization of distinct tumor material that is amenable to generation of viable cell lines. Here, we have characterized 2 novel CRC GEMMs that present with a very low multiplicity of large advanced primary colonic tumors, making these an ideal basis for derivation of cell lines to incorporate into our GEMM-derived orthotopic model. We used primary colonic tumor tissue from these 2 GEMMs to successfully generate low-passage cell lines that recapitulate many canonical features of human CRC. In addition, low-passage cell lines derived from primary tumors recapitulate this enhanced proliferative phenotype when propagated in vitro, as well as in subcutaneous space in vivo. Gene expression signatures generated from these low passage Kras-mutant cell lines are highly concordant with several signatures associated with KRAS mutation, as well as enhanced proliferation, metastasis, and stem-cell signatures, providing evidence for common biology between our model system and CRC. Furthermore, our CRC GEMM expression signatures are highly enriched for biologic functions and processes associated with human cancer, gastrointestinal disease, and altered metabolism. Taken together, these findings suggest that the cell lines derived from primary GEMM tumors recapitulate many canonical features of human CRC biology and are a robust foundation for our orthotopic model.

Although traditional xenograft models suffer from complete species mismatch in the tumor microenvironment, our strategy permits engraftment into immunocompetent, syngeneic hosts. Multiple approaches to develop orthotopic transplant models in mice have been described, including cecal and colonic injections (30–32). Unfortunately, these approaches require subepithelial injection of tumor cells into the supporting stroma, which is not the native microenvironment for CRC carcinogenesis. Here, we describe a method in which we disrupt the native colonic epithelium, thereby permitting robust and reproducible cell line engraftment in the colonic epithelial compartment. Furthermore, our model allows for the development and reimplantation of syngeneic tumor cells using any number of genetic combinations. Limitations intrinsic to the model itself may include a lack of genetic heterogeneity due to the finite number of initial driver mutations built into the model, as well as potential for the tumors themselves to manifest a limited level of intratumoral heterogeneity, as compared with human CRC counterparts. To better recapitulate such heterogeneity, implantation of a mixture of primary cell cultures that derive from independent tumor models could be implemented in the future. Nonetheless, we have developed a platform that allows for the validation of findings from high-throughput in vitro screens in an in vivo setting that recapitulates CRC carcinogenesis in its native environment.

Given the lack of success in generating selective pharmacologic agent against oncogenic KRAS, several efforts have attempted to identify potential synthetic lethal targets (33–36). To this end, the Kras wild-type and mutant models of CRC described and characterized herein will provide a platform for future functional screening efforts aimed at identifying and, more critically, validating (37, 38) such synthetic lethal targets in Kras-mutant CRC. In addition, the ability to transplant these low-passage cell lines back into the relevant colonic microenvironment and reconstitute a colorectal tumor will provide an invaluable platform for future functional screening efforts to identify putative therapeutic targets using RNAi or genetically engineered gain-of-function approaches. Furthermore, we have shown the feasibility of asking sophisticated hypothesis-driven gain-of-function and loss-of-function questions using relatively simple in vitro genetic manipulations that can be rapidly validated in vivo. In this manner, we can circumvent some of the limitations of traditional CRC GEMMs that require laborious targeting of embryonic stem cells and/or cumbersome mouse breeding.

Activation of oncogenes and/or loss of tumor suppressor genes such as KRAS (39), MYC (40), and TP53 (41) increase glucose uptake and lactate production. These observations are consistent with the “Warburg effect” that stipulates that tumor cells rely mainly on aerobic glycolysis to generate ATP instead of the metabolically more efficient mitochondrial oxidative phosphorylation (12). Therefore, mutations in diverse oncogenic pathways may affect glucose use by tumor cells. Consequently, FDG-PET has been used as a diagnostic tool to detect metabolically active foci, which are representative of cancer cell dissemination in the clinical setting (42). Given the prevalence of metabolic pathway enrichment in our transcriptome analysis, we wished to determine whether the activation of oncogenic Kras enhanced metabolic activity in our model system. In support of an altered metabolic state, our functional analyses found that Kras-mutant cell lines show decreased in vitro OCR/ECAR ratios, increased in vitro and in vivo FDG-PET signal, and increased in vitro lactate production. These findings are consistent with a metabolic shift towards increased aerobic glycolysis, termed the Warburg effect (43), and are concordant with recent findings implicating a role for oncogenic KRAS in a shift toward increased glycolysis and glucose consumption (44). This shift in metabolism is thought to provide cancer cells with a growth advantage in the tumor microenvironment, (45), and it has been suggested that aerobic glycolysis can contribute to malignant transformation (46). Furthermore, this finding highlights the potential of FDG-PET for noninvasive discrimination between Kras-mutant and non-mutant CRC metastases. Thus, our Kras-mutant GEMM model presents with several phenotypes of Kras-mutant CRC, including enhanced proliferation, heightened signaling through MAPK and PI3K pathways, and an increased propensity towards aerobic metabolism.

In conclusion, we present here the establishment of 2 genetically defined models of sporadic CRC biology,
including one that displays several hallmark traits of KRAS-driven CRC.

These findings provide evidence that this model system faithfully recapitulates human CRC, thereby making it a highly desirable platform for preclinical therapeutic target identification for KRAS CRC and subsequent in vivo validation.

Disclosure of Potential Conflicts of Interest

S. Weinrich has ownership interest (including patents) in Pfizer and M. V. Heiden is a consultant/advisory board member of Agios Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: E.S. Martin, P.J. Belmont, M.J. Sinnamon, J. Roper, J. Xie, J. Lamb. K.E. Hung


Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): E.S. Martin, P.J. Belmont, M.J. Sinnamon, J. Roper, L. Lee, P. Heidari, S.Y. Lunt, C. Ji, R.T. Bronson, K.E. Hung


Writing, review, and/or revision of the manuscript: E.S. Martin, P.J. Belmont, P.J. Belmont, M.J. Sinnamon, J. Yuan, E.M. Coffee, P. Heidari, S.Y. Lunt, G. Goel, J. Xie, J. Lamb, S. Weinrich, J.L.C. Kan, U. Mahmood, K.E. Hung

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): E.S. Martin, P.J. Belmont, I.C. Richard, L. Lee, S. Weinrich, K.E. Hung


Acknowledgments

The authors thank Dr. Sabine Tejparr for critical review of this manuscript.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked

Disclosure in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 26, 2012; revised January 16, 2013; accepted January 28, 2013; published OnlineFirst February 12, 2013.

References


