KRAS Mouse Models: Modeling Cancer Harboring KRAS Mutations

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Abstract

KRAS is a potent oncogene and is mutated in about 30% of all human cancers. However, the biological context of KRAS-dependent oncogenesis is poorly understood. Genetically engineered mouse models of cancer provide invaluable tools to study the oncogenic process, and insights from KRAS-driven models have significantly increased our understanding of the genetic, cellular, and tissue contexts in which KRAS is competent for oncogenesis. Moreover, variation among tumors arising in mouse models can provide insight into the mechanisms underlying response or resistance to therapy in KRAS-dependent cancers. Hence, it is essential that models of KRAS-driven cancers accurately reflect the genetics of human tumors and recapitulate the complex tumor-stromal intercommunication that is manifest in human cancers. Here, we highlight the progress made in modeling KRAS-dependent cancers and the impact that these models have had on our understanding of cancer biology. In particular, the development of models that recapitulate the complex biology of human cancers enables translational insights into mechanisms of therapeutic intervention in KRAS-dependent cancers.

Keywords: mouse models harboring KRAS mutation, tumor genetics, tumor context, interpatient tumor variation

KRAS Activation in Cancer

GGA GCT GAT GGC GTA often spells misfortune for patients with a cancer diagnosis. Patients with tumors harboring the above sequence have a mutation in the KRAS gene (G12D) that leads to constitutive activation of this potent oncogene. KRAS was identified as an oncogene in 1982^{1,2} and is found to be mutated at a high frequency in human cancers including 95% of pancreatic ductal adenocarcinomas (PDAC), 50% of colon cancers, and 30% of non-small cell lung cancers (NSCLC).^{2,3} Overall, activating mutations in RAS are found in 32% of human cancers, including 21% with KRAS mutation, 8% with N-RAS mutation, and 3% with H-RAS mutation.²

Mutational activation of KRAS results in aggressive cancers, is generally correlated with poor prognosis in cancers, and is associated with poor response to many existing therapies.^{4–6} In fact, targeted therapies of the epidermal growth factor receptor (EGFR) are contraindicated in colon tumors bearing KRAS mutation.⁷ In the absence of KRAS mutations, therapies specifically targeting other oncogenes that are mutationally activated in

cancer have proven to be clinically successful and have led to prolonged patient survival. For example, imatinib, a small molecule inhibitor of the Bcr-Abl oncoprotein, is approved for the treatment of chronic myelogenous leukemia,⁸ and erlotinib and gefitinib, small molecule inhibitors of the EGFR, have shown great promise in the treatment of NSCLC.⁹ However, despite the early recognition of KRAS as an oncogene, efforts to develop therapies targeting KRAS and KRAS-driven tumors have been largely unsuccessful. The frequency of KRAS activation in human cancers combined with the lack of targeted therapy for KRAS highlights the need to identify the Achilles heel(s) of these tumors for therapeutic intervention.

KRAS is a guanine nucleotide transferase that can link cell surface receptors to intracellular signaling pathways including kinase cascades such as the MAPK pathway, lipid kinases such as phosphoinositide 3-kinase (PI3K), and other small molecular weight GTPases including Ral, Rac, and Rho.¹⁰ Through these effectors, KRAS regulates numerous processes including cell proliferation, survival, differentiation, migration, and

extracellular communication. Numerous attempts have been made to directly target the biochemical function of KRAS including molecules that attempt to interfere with the subcellular localization of KRAS (farnesyl transferase inhibitors) or the GTPase activity of the protein.^{11,12} These efforts have met with little success.¹³ More recent efforts have sought to target either downstream components of KRAS-dependent signaling¹⁰ or to identify other proteins whose inhibition is lethal in the context of a KRAS mutation, so-called synthetic lethal targets.¹⁴ Effectively targeting downstream pathways in cancers, or identifying the targets whose inhibition will be synthetic lethal in KRAS-driven tumors, requires a greater depth of understanding of the genetic and cellular context of KRAS activation in human cancers. In light of the multitude of effects of KRAS, including intracellular and intercellular interactions, it is critical to understand KRAS-driven

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Table 1. Key Features of Mouse Models of Cancer

Genetics	Genetics refers to the use of the cancer-relevant oncogenes, tumor suppressor genes, and their mutations in models of cancer for a given tissue.
Context	Context can be defined both genetically and spatially at the cellular and tissue level. Genetic context is defined by the mutations and epigenetic changes present in cells in which an oncogene is activated. Context will affect the outcome of that activating event. Cellular context is defined by the cell type in which any oncogene is activated, and tissue or microenvironmental context is defined by the cells surrounding that in which the initiating mutation is acquired. Importantly, human sporadic tumors develop from a few cells that have lost growth control in a setting of essentially normal cells. As a consequence of tumorigenesis, an intricate signaling network develops between the tumor cells and the normal tumor surroundings, which itself becomes altered in its growth characteristics, and contributes to formation of the tumor proper. In addition, species context is emerging as an important factor, as many host-tumor circuits exhibit species-specific barriers.
Variation	Variation in human tumors is a product of differences in genetic, epigenetic, and microenvironmental contributions to the etiology of any individual tumor. This variation in turn underlies differential patient response to therapy even in cases where one would predict a similar outcome based upon mutation of the therapeutic target. Deregulation of 8 to 10 key pathways that control proliferation, for example, adhesion, apoptosis, migration, differentiation, cell cycle, and survival, is required for a malignant tumor to develop, and individual tumors will achieve these alterations through different mechanisms, resulting in variation among tumors driven by the same signature alterations.

tumorigenesis in a setting that recapitulates the complex biology of tumors in patients. Without this understanding, it is exceedingly difficult to identify relevant modifiers or effectors of KRAS function. Genetically engineered mouse models (GEMMs) of cancer have proven to be valuable tools in cancer research through their capacity to effectively capture the complexity of cancer biology in humans and thereby facilitate greater understanding of the processes involved in tumor etiology.

Modeling Cancer in Mice

The hallmarks of cancer as a disease are genetics, context, and variation (Table 1). These then are the key aspects of any cancer that must be recapitulated in a mouse in order to effectively model the role of any gene in that cancer. For modeling purposes, genetics refers to the use of the cancer-relevant oncogenes, tumor suppressor genes, and their mutations in models of cancer. It is possible to effectively generate mouse models of cancer using genes that do not necessarily recapitulate the genetics of the specific human cancer under study. For example, early mouse models of RAS-driven

cancers were generated using activated versions on the H-RAS gene.¹⁵ While these models were valuable in understanding various cancer-relevant processes, we now understand that there may be tissue-specific bases for the selection of KRAS, N-RAS, or H-RAS for mutational activation, suggesting that tissue-specific contexts exist in which each of these oncoproteins are competent for transformation.¹⁶⁻¹⁹ In addition, subtle differences in downstream effects could significantly alter tumor etiology, and it is now understood that KRAS, N-RAS, and H-RAS can effect different downstream signals.^{20,21} Furthermore, the KRAS gene is mutated or amplified in human cancers. The nature of the mutation utilized in mouse models should reflect that found in human tumors; for example, exon 2 mutations particularly at codon 12 and 13 are the most common KRAS mutations in human cancers and therefore in most cases will reflect the human disease adequately. Similarly, the HER2 receptor is a powerful oncoprotein in human breast cancer and a useful therapeutic target.²² However, mouse models of human breast cancer are often generated using an activated version of HER2

or its mouse or rat orthologs (ErbB2, Neu). This mutation was originally identified in the rat ERBB virus²³ but is rarely found in human cancers.^{24,25} Generation of a mouse model of breast tumors with this activated Neu/HER2 gene resulted in models that have a short latency, have high penetrance, and are effective tools that have led to significant advances in our understanding of breast cancer.²⁶ Moreover, it is possible that with the advent of more sensitive sequencing procedures, similar activating mutations may be found among the amplified copies of HER2 in human cancers.^{27,28} However, the impact of HER2 targeted therapies such as Herceptin or lapatinib on model systems is likely to be altered by the use of this activated HER2 allele and may not recapitulate the clinical experience in treating human tumors. Hence, it may not be possible to meaningfully assess the impact of current or novel therapies on cancers driven by the human cancer-relevant HER2 alteration or its interacting partners in a mouse model driven by an oncogene activated in a manner that is inconsistent with human tumors. Conversely, the BRAF gene is mutationally activated in approximately 60% of human melanomas.²⁹ However, attempts to generate mouse models of malignant melanoma using the human-relevant activated mutant of BRAF (V600E) have had limited success, and combination with engineered loss of the PTEN tumor suppressor was required for progression to malignancy.³⁰ This suggests an essential function for additional events beyond activation of the predominant oncogene in the etiology of malignant melanoma. It is clear that the context in which an oncogene is activated will affect the outcome of that activating event.

Context can be defined both genetically and spatially and at the cellular and tissue level. As discussed above, additional genetic alterations identified in human melanoma may be prerequisites for melanomagenesis by activated BRAF,^{29,30} thus defining the genetic context in which this oncogene is competent

for transformation. Cellular context is defined by the cell in which any oncogene is activated, and the tissue or micro-environmental context is defined by the cells surrounding that in which the initiating mutation is acquired. Importantly, human sporadic tumors develop from a few cells that have lost growth control in a setting of essentially normal cells.³¹ As a consequence of tumorigenesis, an intricate signaling network develops between the tumor cells and this normal tumor milieu, which itself becomes altered in its growth characteristics,³² and contributes to formation of the tumor proper. This is in contrast with transgenic animals that express an oncogene in a specified cell type or tissue. In these animals, the oncogene is expressed in all cells in the tissue in question, and tumors form from cells that grow in the vicinity of cells that have altered growth regulation from the start. This phenomenon has been referred to as “cancerization of the field.”³¹⁻³⁴ The signaling network that will form in such a tumor is most likely different from human primary tumors. Studies in transgenic mice have demonstrated that it is possible to recapitulate tumor-stroma crosstalk in mouse models. For example, in a mouse model of prostate cancer, the tumor cells exert a selective pressure on surrounding fibroblasts to undergo loss of p53 and subsequently increased proliferation to support tumor growth.³⁵ The location at which a tumor forms helps to define the tumor microenvironment, and this in turn helps to define the tumor-stromal signaling network. It is easy to see, for example, that cancer cell lines growing in the subcutaneous space of mice, albeit useful tools in many respects, are not suited to guide our understanding of the intricate interrelationship of tumor cells and stroma that is manifest during tumorigenesis in tissues of human patients.

KRAS GEMMs: Milestones in Modeling Cancer

As discussed above, H-RAS was one the first oncogenes cloned from oncogenic

Table 2. Considerations for selection of appropriate GEMMs

Model	Key feature	Considerations
Transgenic and gene knockout models	Expression or deletion of genes in target tissues	Simple, well-established technologies; accurate genetics; complex breeding required; cancerization of target tissue
Inducible models	Inducible expression or deletion of genes in target tissues	Enables studies of progression, maintenance, and regression; cancerization of target tissue; even more complex breeding
Mosaic models	Deletion in some, but not all, cells of a target tissue	Tumor cells in context of normal host cells; viral delivery can limit target tissues
Chimeric models	Genetic manipulation of embryonic stem cells and transfer into preimplantation embryos	Rapid engineering of complex genetics; high degree of tumor-stromal interaction; no breeding; preimplantation embryo manipulation
Human-in-mouse models	Human primary tissue, which can be transduced, reconstitutes “normal mouse tissue,” and primary tumors develop from the human cells	Tumors arise in human cells; limited tumor-stromal crosstalk due to species incompatibility

retroviruses and was demonstrated to be an effective oncogene in mice in seminal work from Sinn *et al.*¹⁵ MMTV–H-RAS transgenic mice developed mammary gland tumors and along with the earlier MMTV–c-Myc models^{36,37} ushered in the era of modeling tumorigenesis in transgenic mice. However, this model and later germline genetically engineered mouse models share common limitations including the presence of the oncogenic mutation in all cells in the target tissue and the resultant cancerization of the field. Moreover, the use of H-RAS as the oncogene in this model does not address the predominant role of KRAS mutations in human breast cancers harboring activated RAS.³⁸ Subsequently, the field has become increasingly sophisticated in modeling tumorigenesis in the mouse (Table 2). RAS-dependent models have remained at the leading edge of mouse modeling, and a progressive increase in the use of KRAS in models reflects the frequency and importance of KRAS mutations in human tumors. Specific models of RAS-dependent tumorigenesis serve to illustrate the evolution of mouse models of cancer as ever more faithful models of human disease.

The advent of controllable systems in which expression of RAS could be turned on and off added significantly to the scope of biology that could be examined with a mouse model.³⁹ For example, H-RAS under the control of a doxycycline-dependent expression system can induce melanomagenesis in mice^{29,40} and enables the study of tumor initiation, progression, and maintenance. Upon doxycycline withdrawal, H-RAS expression is downregulated. Subsequent events model the course of tumor regression and provide a genetic model of pharmacological inhibition of H-RAS signaling in cancer. Similar studies have also demonstrated the sequelae of events that accompany downregulation of KRAS in a doxycycline-regulated KRAS-dependent model of mammary tumorigenesis.⁴¹ Further investigation of those events associated with regression of KRAS-dependent tumors in mice could provide valuable insights into the tumor maintenance functions that are lost and that might represent attractive targets for therapeutic intervention in KRAS-dependent human cancers.

The ability to regulate oncogene expression expands the scope of cancer

biology that can be examined using mouse models. However, in these models, it is still challenging to overcome the cancerization of the field phenomenon because all cells in the target tissue express the oncogene. Careful dosing of inducers might overcome this to some extent through stochastic activation of the oncogene.^{42,43} However, conditional expression of the KRAS oncogene in mosaic models can be used to overcome this limitation.^{44,45} In the latent lox-STOP-lox KRASG12D (Isl) model, a G12D mutation is introduced in exon 2 of the mouse endogenous KRAS allele. This allele contains a STOP cassette, a yeast gene with multiple polyA signals flanked by loxP sites, prior to exon 2. The STOP cassette silences the expression of the mutant KRAS allele. Tissue- and/or time-dependent expression of Cre recombinase removes the STOP cassette and allows transcription from the mutated KRAS allele. The Isl allele has become the standard for KRAS alleles used in transgenic models and has been applied to many tissue types. Further use of this model is restricted only by the availability of tissue-specific promoters for expression of the recombinase. Alternately, the latent oncogene can be constructed such that it is activated by spontaneous recombination between a wild-type and a mutant allele, a low frequency event.³⁴ The advantage of this approach is that it most closely recapitulates the spontaneous activation of oncogenes that occurs in human cancers. However, the practical disadvantage of such a model is the low frequency of activation and the resulting low penetrance model of the cancer in question. Thus, there is a fine balance to strike between accurately modeling the spontaneous nature of human cancers and creating a model that lends itself to effective thorough study of the oncogenic process.

Chimeric models have recently been described⁴⁶ in which the requisite genetic modifications are introduced into embryonic stem (ES) cells, which in turn are injected into preimplantation embryos.

The resulting chimeric animal constitutes the model, and tumors develop from the descendants of the engineered ES cells, which are mixed with the descendants of the wild-type host embryo. No breeding steps are required. Multiple genetic elements and mutations can be engineered with ease into the ES cells. This generally results in faster model generation times, facilitates the development of complex models, and reduces the overall number of mice required. Importantly, the chimeric nature of the target tissue ensures that tumors develop surrounded by nonengineered normal cells. In this manner, the crosstalk between mutationally activated tumor cells and normal stroma is intrinsic to the model, ensuring a more appropriate context for tumorigenesis. Zhou *et al.* described a KRAS-dependent chimeric model of lung adenocarcinoma in addition to an allelic series of EGFR mutations and a HER2 model for lung adenocarcinoma.⁴⁶ In this model, the tumors harbored high levels of stromal infiltration and architecture resembling human cancers, further highlighting the importance of context in modeling tumor etiology. Interestingly, in this series of lung adenocarcinoma models, the authors could pinpoint a difference in downstream signaling between EGFR- and KRAS-dependent tumors, implicating the JNK pathway in KRAS-dependent lung adenocarcinomas as a potential node of therapeutic intervention.

Modeling human tumors in mice has led to many significant discoveries and insights into the oncogenic process. To further enhance the human relevance of mouse models of cancer, a number of groups have undertaken engineering of primary human tissue, then transplanting the tissue/stem cells orthotopically into mice and allowing primary human tumors to develop in a mouse host tissue. Again, KRAS models were an integral part of this development. Viral transduction of primary human skin to express KRAS(V12) has been used to pioneer a skin carcinoma model.⁴⁷ In a similar fashion, N-RAS also has been used to establish a melanoma model.⁴⁸ A

number of studies demonstrate the reconstruction of mammary gland formation from human mammary tissue in a cleared mouse fat pad.^{49,50} When the donor mammary tissue was transduced by a combination of SV40 and either activated KRAS or HER2, basal cell-like mammary tumors or ductal carcinomas were obtained.⁴⁹ These human-in-mouse (HIM) models have removed the species barrier in modeling tumors and will be invaluable in elucidating genetic dependencies in human KRAS tumors. Unlike the chimeric approach described above, the HIM approach is limited in modeling cellular crosstalk between mouse stroma and human tumor because of the species-specific nature of some of these interactions. While these early HIM models do not fully recapitulate all aspects of human tumor context, further progress in understanding KRAS-dependent tumorigenesis is certain to come from this approach.

KRAS-Dependent Cancer Biology

The currently available KRAS-driven mouse models of cancer have been used to great effect to enhance our understanding of tumor biology. For example, over 90% of PDAC contain activating mutations in KRAS, and numerous studies using KRAS-dependent mouse models of pancreatic cancer have added to our understanding of this highly lethal disease. Pancreas-specific Cre-mediated activation of the mutant KRAS allele (KRAS(G12D)) in mice forms the basis of many of the systems used to model this highly KRAS-dependent disease.^{45,51} Activation of KRAS alone in the pancreatic epithelium led to the development of premalignant ductal neoplasias known as pancreatic intraepithelial neoplasias (PanINs), highlighting the need for additional cooperating events to promote tumor progression to the malignant PDAC stage. In a number of studies, additional genetic alterations have been combined with activation of KRAS to identify cooperating events that may better define the genetic

context of KRAS-dependent pancreatic tumorigenesis. Loss of the tumor suppressors Ink4a/Arf or p53 have been identified as key steps in PDAC progression in humans.⁵²⁻⁵⁴ Pancreas-specific deletion of Ink4a/Arf,⁵¹ deletion of the individual p16^{Ink4a} tumor suppressor,⁵⁵ or mutational inactivation or loss of p53^{55,56} in conjunction with activation of KRAS results in highly aggressive PDAC formation in mice. SMAD4 inactivation occurs in approximately 50% of human PDAC,⁵⁷ and deletion of SMAD4 cooperates with KRAS to enhance progression to ductal adenocarcinoma.^{58,59} KRAS-dependent pancreatic tumors were rendered even more aggressive by the combination with deletion of both Ink4a/Arf and SMAD4,⁵⁸ a model that encompasses a broader range of the mutations found in the human disease.

The combination of KRAS activation with additional key genetic events identified from human pancreatic cancers in a mouse model would be expected to more effectively recapitulate the genetics and etiology of human disease. Consistent with this notion, histopathological analysis of tumors arising from these pancreatic cancer models reflects classic attributes of human disease including the presence of ductal adenocarcinomas, dense fibrous stroma, and expression of characteristic mucins.^{55,56,58}

Additional signaling pathways may impact KRAS-dependent tumorigenesis by affecting other aspects of tumor biology. For example, the RAS and Notch pathways are intimately intertwined during early stages of development⁶⁰⁻⁶² and during tumorigenesis.^{62,63} Moreover, Notch signaling is implicated in the development of pancreatic cancers,^{60,64} and activation of Notch1 can cooperate with activation of KRAS to enhance progression of PanINs to aggressive PDAC.^{65,66} However, it appears that Notch signaling may act at an early stage in pancreatic tumorigenesis because inhibition of Notch signaling by γ -secretase inhibitors is effective at the PanIN stage but less so once tumors have progressed to ductal adenocarcinoma.⁶⁷ How then

does Notch enhance KRAS tumorigenesis? Recent evidence suggests that the cellular context in which KRAS is activated may dictate the nature and degree of tumor progression.⁶⁸ It may be that activation of Notch promotes expansion of the appropriate cellular context in which KRAS is most effective as an oncogene. In this regard, it is noteworthy that Notch promotes a Pdx1-positive progenitor/exocrine fate over endocrine fate in pancreatic development.^{61,69,70} Furthermore, it appears that a subset of Pdx1-positive cells is susceptible to KRAS-dependent tumorigenesis, whereas endocrine cells are refractory to KRAS-dependent transformation.⁶⁸ These studies suggest that the cellular context in which KRAS is activated is a critical modifier of oncogenic potential, and factors, such as Notch signaling, that affect this context will dictate the course of KRAS-dependent oncogenesis. Hence, it is essential to model the appropriate cellular context of KRAS activation to effectively model the disease in question.

Our understanding of the pathways that modify KRAS oncogenicity can be exploited in the development of therapeutics that target pathways that are required for KRAS-dependent tumorigenesis. In this manner, it may be possible to develop synthetic lethal approaches to target KRAS-dependent tumors.^{14,71} Studies in cell culture have sought to identify genes whose expression is required for viability in the context of an activated KRAS signal.⁷²⁻⁷⁴ The identification of synthetic lethal interactions between KRAS dependence and druggable targets such as the receptor tyrosine kinase MST1R,⁷⁴ the tyrosine kinase Syk,⁷⁴ the serine-threonine kinase STK33,⁷³ and components of a PLK1-dependent mitotic pathway, and the proteasome⁷² suggest strategies to target KRAS-dependent cancers. Importantly, some of these targets have been implicated in KRAS-dependent human cancers including PDAC, suggesting that they may be relevant *in vivo* in the human disease setting. For example, the RTK MST1R was identified as a strong

candidate synthetic lethal target from *in vitro* studies.⁷⁴ MST1R is expressed at high levels in later stages of human pancreatic cancers but is not expressed in normal pancreatic epithelium or at early stages such as PanIN1.⁷⁵ Moreover, this expression pattern is recapitulated in a KRASG12D mouse model of pancreatic cancer.⁷⁵ Hence, the development of KRAS-driven cancer models that effectively model human disease may facilitate further *in vivo* study of novel therapeutic targets identified from *in vitro* synthetic lethal screens and provide high-quality models for the rapid testing of the effect of therapeutics directed against such targets on KRAS-dependent tumorigenesis.

NSCLC often harbors KRAS mutations, particularly in adenocarcinomas but also in squamous cell carcinomas. KRAS mutations are frequently associated with smoking and have an unfavorable prognosis. Fisher *et al.*⁷⁶ reported a KRAS adenocarcinoma model overexpressing inducible KRAS with the CCSP promoter. The allelic recombination model³⁴ and the Isl approach have been used to model lung cancer in mice. In the later instance, animals exposed to Cre expression by viral delivery develop adenocarcinomas from the bronchial-alveolar epithelium in the vicinity of normal tissue, thereby preserving tissue context during tumor development. Similarly, chimeric models of lung cancer have been shown to preserve the cellular context and model KRAS-dependent lung adenocarcinoma.⁴⁶ The recent development of EGFR inhibitors and their approval for the treatment of lung cancer lead to a flurry of translational studies with these molecules in mouse models of lung cancer.^{46,77,78} These studies have demonstrated the utility of pre-clinical mouse models in elucidating the different mechanisms that underlie the responses of PI3K- and KRAS-driven lung tumors to clinically relevant EGFR inhibitors.

Mouse models have also been developed to study the role of KRAS in other cancers that have not been discussed

within the confines of this review. These include KRAS-dependent mouse models of colorectal cancer,^{19,79} breast cancer,^{80,81} hepatocellular carcinoma,⁸² and various leukemias.⁸³

Translational Application of KRAS GEMMs

The pathways that are implicated as modifiers or effectors of KRAS-dependent oncogenesis differ between tissues, for example, pancreatic and lung cancers. Moreover, the response to targeted therapeutic agents may differ depending upon the tissue context in which an activated KRAS mutant is found. For example, KRAS mutation appears to inhibit response to EGFR targeting monoclonal antibodies in colon cancer.^{5,84,85} However, this may not be the case in human pancreatic cancers where 95% of patients harbor KRAS mutations, and a significant portion of patients respond to EGFR inhibitors.^{86,87} These observations further support the role of tissue context in modifying the effects of KRAS mutation and in altering the genetic environment in which a KRAS mutation acts. The role of the tumor milieu in modifying response to therapeutic approaches, as evidenced by these examples, remains to be understood.

The evolution of KRAS-dependent mouse models of cancer illustrates the progression of GEMMs in general toward capturing the complexity of human tumorigenesis. This evolution has enabled more effective study of specific aspects of cancer biology *in vivo* including interactions among genetic events and the interdependency of tumor cells and their microenvironment. However, it is difficult to effectively capture the intrinsic variation of human cancer in genetically engineered mouse models of a specific tumor type. Variation in human tumors is a product of differences in genetic, epigenetic, and microenvironmental contributions to the etiology of any individual tumor. This variation in turn underlies differential patient response to therapy even in cases

where one would predict a similar outcome based upon mutation of the therapeutic target. For example, patients harboring identical activating mutations in EGFR respond differently to therapies directed against this receptor.⁸⁸ Various explanations have been proposed for this *a priori* variation in response, including the presence of additional mutations in EGFR that confer resistance to the drug,^{89,90} activity of alternate pathways that may bypass or reduce dependence upon EGFR (e.g., HGF/MET),⁷⁸ or unidentified changes in tumor genetics or the microenvironment that affect drug response and alter the epithelial-mesenchymal state of tumor cells.⁹¹ Perhaps the longest standing example of variation in a single tumor type leading to variation in drug response is the effect of the monoclonal antibody trastuzumab on HER2-positive breast cancer.⁹² In this case, not all patients bearing HER2-amplified breast cancers respond to the same degree to treatment. The central role of tumor variation in understanding differential response to therapy suggests that it is critical to develop model systems to account for genetic, epigenetic, and microenvironmental variation among individual tumors.

Variation in GEMMs

How can the intrinsic variation in human cancers be captured and modeled in mice? The use of large cohorts of individual mice bearing identical engineered oncogenic lesions can be used to assess the ability of GEMMs to predict clinical outcomes. For example, a series of alleles of a specific therapeutic target that contain various mutations hypothesized to influence outcome can be engineered into GEMMs and tested for response to therapy. This targeted approach is particularly applicable to well-understood targeted therapeutics, for example, testing gefitinib or erlotinib in a series of EGFR mutant alleles to understand the influence of various different mutations on response.^{46,93} Alternately, putative modifiers of drug

response could be engineered into large cohorts of mice to examine the effect on therapies targeted to a specific target. To investigate the effect of KRAS on response to current therapies in the lung and pancreas, mutant KRAS mice were utilized to test treatment of lung and pancreas tumors with the tyrosine kinase inhibitor erlotinib and an anti-mouse VEGF antibody or inhibitors of HSP90 function.^{94,95} The use of GEMMs was highly predictive of response to treatment when the treatment regimen in mice was comparable to that used in human patients. Hence, it appears to be feasible to model the effect of KRAS mutation and other genetic alterations on drug response in human cancers. However, this approach is predicated on *a priori* knowledge of the key genetic differences between tumors in order to generate individual models that capture that specific variation found in human patients. Therefore, studies of this nature can be used to test and confirm hypotheses derived from clinical observations of drug response. The limitation of such studies is that they do not necessarily encompass the full repertoire of mutations in any population of tumors that may affect drug response (e.g., KRAS tumors do not represent the entire population of lung adenocarcinomas), and they do not provide an opportunity to identify hitherto unknown variation that may account for differential response to therapy. Extensive characterization of the genetic, epigenetic, and histological variation among the tumors prior to treatment is mutually exclusive with treatment. This precludes direct correlation of variation among tumors with subsequent response and thus impedes the ability of GEMMs to identify *de novo* the characteristic variation that underlies response or resistance to a particular therapy.

It may be possible to capture variation more broadly without *a priori* knowledge of the source of that variation using an archive of tumors generated from a single GEMM. Dereglulation of 8 to 10 key pathways that control proliferation,

adhesion, apoptosis, migration, differentiation, cell cycle, and survival is required for a malignant tumor to develop.^{96,97} Similarly, the stochastic nature of tumorigenesis in GEMMs is consistent with the notion that activation of multiple pathways is required in addition to the engineered lesions that underlie each model. The initial latency period prior to tumorigenesis in any GEMM allows time to achieve the requisite alterations, including acquisition of a series of secondary genetic events by each individual tumor and remodeling of the tumor microenvironment. Moreover, individual tumors will achieve these alterations through different mechanisms, resulting in variation among tumors driven by the same signature alterations that were engineered into the GEMM. For example, activation of the PI3K pathway appears to be critical in tumorigenesis in many settings.⁹⁸ There are multiple alterations that may result in activation of this pathway, including for example, deletion of PTEN, TSC1, TSC2, and LKB; mutation of p110a; and amplification or mutation of EGFR, HER2, MET, or IGF1R. None of these alterations will solely influence the activity of the PI3K pathway, and those additional alteration-specific effects will lead to significant variation in signaling and resultant tumor biology. Intertumor variation among individual tumors from the same GEMM has been well described using genomic approaches such as expression profiling and array comparative genomic hybridization.⁹⁹⁻¹⁰³ These events in GEMMs are similar in nature to those that contribute to variation in human tumorigenesis.^{29,104} Therefore, if the appropriate driving oncogenic lesions (e.g., KRAS(G12D) mutation) underpin the model, then the differences between individual tumors in acquired secondary genetic alterations, and in crosstalk between the tumor and the microenvironment, are likely to encompass much of the variation in these characteristics that underlie differences in the response of human cancers to therapy.

An interesting approach to exploiting the spontaneously arising variation in

GEMMs has been described recently.¹⁰⁵ An archive of 107 primary HER2-positive mammary gland tumors was generated by minimal propagation of the primary tumors in immune-compromised mice. The resultant archive of tumors was then characterized by gene expression profiling in the absence of any treatment. Independently, the archived tumors were subjected to drug treatment, and responsive and nonresponsive tumors were identified empirically. Comparison of the gene expression profiles of responsive and nonresponsive tumors enabled identification of expression markers that may predict response. In this particular study, a gene signature was identified that correlated with response of HER2-driven mammary tumors to a γ -secretase inhibitor. Such preclinical studies could then be extended to generate translational hypotheses pertaining to biomarkers that might predict response in HER2-positive breast cancers in a clinical setting. Given the plethora of KRAS-driven mouse models, it is feasible to apply a similar approach to elucidate a genetic signature that identifies KRAS-driven cancers that are sensitive to a specific therapy. In fact, a KRAS-dependent NSCLC archive has been generated using a chimeric model approach (Y. Zhou *et al.*, unpublished results). The archived chimeric tumors retain the characteristics of the primary tumors, including high degrees of stromal infiltration. In addition, an archive of KRAS-dependent mammary tumors has been established from the mammary gland HIM transplantation model (M. Wu *et al.*, unpublished results). Similarly, Jimeno *et al.* have developed an archive of primary patient explants for pancreatic tumors.¹⁰⁶ These novel tumor archives, with their inherent variation, represent another valuable platform for studies of drug response and resistance that will extend our understanding of KRAS function in cancer.

Summary

KRAS GEMMs have been of tremendous value to cancer research and our understanding of the biology of tumors

harboring a KRAS mutation. The intractability of KRAS by conventional therapeutics presents unique challenges for translational research. The need to interfere indirectly with tumor survival in KRAS mutated tumors highlights the need for careful modeling of all aspects of KRAS-dependent cancers. This holds these models to a high standard in adhering to the principles of genetics, context, and variation in disease modeling (Table 1). Certainly, elucidating the genetic context in which KRAS mutations can become dominant, as well as understanding the accompanying tumor-stromal interactions, will be of the highest priority in searching for nodes of therapeutic intervention in KRAS-dependent tumors. In addition, understanding the inherent variation among tumors that underlies differential drug response and identifying the genetic causes of this variation should be a high priority for KRAS-dependent mouse models of cancer.

No single approach to modeling cancer in the mouse recapitulates all of the key aspects of human disease. However, careful selection of the optimal approach for the question at hand, and understanding of the limitations of the model used, will continue to enhance our understanding of cancer biology and will help guide future clinical development.

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References

1. Der CJ, Cooper GM. Altered gene products are associated with activation of cellular rasK genes in human lung and colon carcinomas. *Cell*. 1983;32:201-8.

2. Bos JL. ras oncogenes in human cancer: a review. *Cancer Res.* 1989;49:4682-9.
3. Chang EH, Gonda MA, Ellis RW, Scolnick EM, Lowy DR. Human genome contains four genes homologous to transforming genes of Harvey and Kirsten murine sarcoma viruses. *Proc Natl Acad Sci U S A.* 1982;79:4848-52.
4. Ueberall I, Kolar Z, Trojanec R, Berkovcova J, Hajdich M. The status and role of ErbB receptors in human cancer. *Exp Mol Pathol.* 2008;84:79-89.
5. Cappuzzo F, Varella-Garcia M, Finocchiaro G, *et al.* Primary resistance to cetuximab therapy in EGFR FISH-positive colorectal cancer patients. *Br J Cancer.* 2008;99:83-9.
6. Eberhard DA, Johnson BE, Amler LC, *et al.* Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. *J Clin Oncol.* 2005;23:5900-9.
7. Amado RG, Wolf M, Peeters M, *et al.* Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol.* 2008;26:1626-34.
8. Druker BJ. STI571 (Gleevec) as a paradigm for cancer therapy. *Trends Mol Med.* 2002;8:S14-8.
9. Tamura K, Fukuoka M. Gefitinib in non-small cell lung cancer. *Expert Opin Pharmacother.* 2005;6:985-93.
10. Downward J. Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer.* 2003;3:11-22.
11. Kohl NE, Omer CA, Conner MW, *et al.* Inhibition of farnesyltransferase induces regression of mammary and salivary carcinomas in ras transgenic mice. *Nat Med.* 1995;1:792-7.
12. Friday BB, Adjei AA. K-ras as a target for cancer therapy. *Biochim Biophys Acta.* 2005;1756:127-44.
13. Johnson BE, Heymach JV. Farnesyl transferase inhibitors for patients with lung cancer. *Clin Cancer Res.* 2004;10:4254s-7s.
14. Sawyers CL. Finding and drugging the vulnerabilities of RAS-dependent cancers. *Cell.* 2009;137:796-8.
15. Sinn E, Muller W, Pattengale P, Tepler I, Wallace R, Leder P. Coexpression of MMTV/v-Ha-ras and MMTV/c-myc genes in transgenic mice: synergistic action of oncogenes in vivo. *Cell.* 1987;49:465-75.
16. To MD, Wong CE, Karnezis AN, Del Rosario R, Di Lauro R, Balmain A. Kras regulatory elements and exon 4A determine mutation specificity in lung cancer. *Nat Genet.* 2008;40:1240-4.
17. Berns A. Kras and Hras: what is the difference? *Nat Genet.* 2008;40:1149-50.
18. Quinlan MP, Quatela SE, Philips MR, Settleman J. Activated Kras, but not Hras or Nras, may initiate tumors of endodermal origin via stem cell expansion. *Mol Cell Biol.* 2008;28:2659-74.
19. Haigis KM, Kendall KR, Wang Y, *et al.* Differential effects of oncogenic K-Ras and N-Ras on proliferation, differentiation and tumor progression in the colon. *Nat Genet.* 2008;40:600-8.
20. Parikh C, Subrahmanyam R, Ren R. Oncogenic NRAS, KRAS, and HRAS exhibit different leukemogenic potentials in mice. *Cancer Res.* 2007;67:7139-46.
21. Fotiadou PP, Takahashi C, Rajabi HN, Ewen ME. Wild-type NRas and KRas perform distinct functions during transformation. *Mol Cell Biol.* 2007;27:6742-55.
22. Nielsen DL, Andersson M, Kamby C. HER2-targeted therapy in breast cancer: monoclonal antibodies and tyrosine kinase inhibitors. *Cancer Treat Rev.* 2009;35:121-36.
23. Ursini-Siegel J, Schade B, Cardiff RD, Muller WJ. Insights from transgenic mouse models of ERBB2-induced breast cancer. *Nat Rev Cancer.* 2007;7:389-97.
24. Kwong KY, Hung MC. A novel splice variant of HER2 with increased transformation activity. *Mol Carcinog.* 1998;23:62-8.
25. Saya H, Ara S, Lee PS, Ro J, Hung MC. Direct sequencing analysis of transmembrane region of human Neu gene by polymerase chain reaction. *Mol Carcinog.* 1990;3:198-201.
26. Moody SE, Sarkisian CJ, Hahn KT, *et al.* Conditional activation of Neu in the mammary epithelium of transgenic mice results in reversible pulmonary metastasis. *Cancer Cell.* 2002;2:451-61.
27. McKean-Cowdin R, Kolonel LN, Press MF, Pike MC, Henderson BE. Germ-line HER-2 variant and breast cancer risk by stage of disease. *Cancer Res.* 2001;61:8393-4.
28. Finkle D, Quan ZR, Asghari V, *et al.* HER2-targeted therapy reduces incidence and progression of midlife mammary tumors in female murine mammary tumor virus huHER2-transgenic mice. *Clin Cancer Res.* 2004;10:2499-511.
29. Chin L. The genetics of malignant melanoma: lessons from mouse and man. *Nat Rev Cancer.* 2003;3:559-70.
30. Dankort D, Curley DP, Carlidge RA, *et al.* Braf(V600E) cooperates with Pten loss to induce metastatic melanoma. *Nat Genet.* 2009;41:544-52.
31. Garcia SB, Park HS, Novelli M, Wright NA. Field cancerization, clonality, and epithelial stem cells: the spread of mutated clones in epithelial sheets. *J Pathol.* 1999;187:61-81.
32. Deng G, Lu Y, Zlotnikov G, Thor AD, Smith HS. Loss of heterozygosity in normal tissue adjacent to breast carcinomas. *Science.* 1996;274:2057-9.
33. Berns A. Cancer: improved mouse models. *Nature.* 2001;410:1043-4.
34. Johnson L, Mercer K, Greenbaum D, *et al.* Somatic activation of the K-ras oncogene causes early onset lung cancer in mice. *Nature.* 2001;410:1111-6.
35. Hill R, Song Y, Cardiff RD, Van Dyke T. Selective evolution of stromal mesenchyme with p53 loss in response to epithelial tumorigenesis. *Cell.* 2005;123:1001-11.
36. Leder A, Pattengale PK, Kuo A, Stewart TA, Leder P. Consequences of widespread deregulation of the c-myc gene in transgenic mice: multiple neoplasms and normal development. *Cell.* 1986;45:485-95.
37. Stewart TA, Pattengale PK, Leder P. Spontaneous mammary adenocarcinomas in transgenic mice that carry and express MTV/myc fusion genes. *Cell.* 1984;38:627-37.
38. Bamford S, Dawson E, Forbes S, *et al.* The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website. *Br J Cancer.* 2004;91:355-8.
39. Chin L, DePinho RA. Flipping the oncogene switch: illumination of tumor maintenance and regression. *Trends Genet.* 2000;16:147-50.
40. Chin L, Tam A, Pomerantz J, *et al.* Essential role for oncogenic Ras in tumour maintenance. *Nature.* 1999;400:468-72.
41. Podsypanina K, Politi K, Beverly LJ, Varmus HE. Oncogene cooperation in tumor maintenance and tumor recurrence in mouse mammary tumors induced by Myc and mutant Kras. *Proc Natl Acad Sci U S A.* 2008;105:5242-7.
42. Barker N, Ridgway RA, van Es JH, *et al.* Crypt stem cells as the cells-of-origin of intestinal cancer. *Nature.* 2009;457:608-11.
43. Sato T, Vries RG, Snippert HJ, *et al.* Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature.* 2009;459:262-5.
44. Jackson EL, Willis N, Mercer K, *et al.* Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. *Genes Dev.* 2001;15:3243-8.
45. Tuveson DA, Shaw AT, Willis NA, *et al.* Endogenous oncogenic K-ras(G12D) stimulates proliferation and widespread neoplastic and developmental defects. *Cancer Cell.* 2004;5:375-87.
46. Zhou Y, Rideout WM, 3rd, Zi T, *et al.* Chimeric mouse tumor models reveal differences in pathway activation between ERBB family- and KRAS-dependent lung adenocarcinomas. *Nat Biotechnol.* 2010;28:71-8.
47. Lazarov M, Kubo Y, Cai T, *et al.* CDK4 coexpression with Ras generates malignant human epidermal tumorigenesis. *Nat Med.* 2002;8:1105-14.
48. Chudnovsky Y, Adams AE, Robbins PB, Lin Q, Khavari PA. Use of human tissue to assess the oncogenic activity of melanoma-associated mutations. *Nat Genet.* 2005;37:745-9.
49. Wu M, Jung L, Cooper AB, *et al.* Dissecting genetic requirements of human breast tumorigenesis in a tissue transgenic model of human breast cancer in mice. *Proc Natl Acad Sci U S A.* 2009;106:7022-7.
50. Kuperwasser C, Chavarria T, Wu M, *et al.* Reconstruction of functionally normal and malignant human breast tissues in mice. *Proc Natl Acad Sci U S A.* 2004;101:4966-71.
51. Aguirre AJ, Bardeesy N, Sinha M, *et al.* Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. *Genes Dev.* 2003;17:3112-26.
52. Bardeesy N, DePinho RA. Pancreatic cancer biology and genetics. *Nat Rev Cancer.* 2002;2:897-909.
53. Bardeesy N, Sharpless NE, DePinho RA, Merlino G. The genetics of pancreatic adenocarcinoma: a roadmap for a mouse model. *Semin Cancer Biol.* 2001;11:201-18.
54. Hezel AF, Kimmelman AC, Stanger BZ, Bardeesy N, DePinho RA. Genetics and biology of pancreatic ductal adenocarcinoma. *Genes Dev.* 2006;20:1218-49.
55. Bardeesy N, Aguirre AJ, Chu GC, *et al.* Both p16(Ink4a) and the p19(Arf)-p53 pathway constrain progression of pancreatic adenocarcinoma in the mouse. *Proc Natl Acad Sci U S A.* 2006;103:5947-52.
56. Hingorani SR, Wang L, Multani AS, *et al.* Trp53R172H and KrasG12D cooperate to promote

- chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. *Cancer Cell*. 2005;7:469-83.
57. Hansel DE, Kern SE, Hruban RH. Molecular pathogenesis of pancreatic cancer. *Annu Rev Genomics Hum Genet*. 2003;4:237-56.
58. Bardeesy N, Cheng KH, Berger JH, *et al*. Smad4 is dispensable for normal pancreas development yet critical in progression and tumor biology of pancreas cancer. *Genes Dev*. 2006;20:3130-46.
59. Izeradjene K, Combs C, Best M, *et al*. Kras(G12D) and Smad4/Dpc4 haploinsufficiency cooperate to induce mucinous cystic neoplasms and invasive adenocarcinoma of the pancreas. *Cancer Cell*. 2007;11:229-43.
60. De La OJ, Murtaugh LC. Notch signaling: where pancreatic cancer and differentiation meet? *Gastroenterology*. 2009;136:1499-502.
61. Murtaugh LC, Stanger BZ, Kwan KM, Melton DA. Notch signaling controls multiple steps of pancreatic differentiation. *Proc Natl Acad Sci U S A*. 2003;100:14920-5.
62. Sundaram MV. The love-hate relationship between Ras and Notch. *Genes Dev*. 2005;19:1825-39.
63. Fitzgerald K, Harrington A, Leder P. Ras pathway signals are required for notch-mediated oncogenesis. *Oncogene*. 2000;19:4191-8.
64. Mysliwiec P, Boucher MJ. Targeting Notch signaling in pancreatic cancer patients: rationale for new therapy. *Adv Med Sci*. 2009;54:136-42.
65. De La OJ, Emerson LL, Goodman JL, *et al*. Notch and Kras reprogram pancreatic acinar cells to ductal intraepithelial neoplasia. *Proc Natl Acad Sci U S A*. 2008;105:18907-12.
66. De La OJ, Murtaugh LC. Notch and Kras in pancreatic cancer: at the crossroads of mutation, differentiation and signaling. *Cell Cycle*. 2009;8:1860-4.
67. Plentz R, Park JS, Rhim AD, *et al*. Inhibition of gamma-secretase activity inhibits tumor progression in a mouse model of pancreatic ductal adenocarcinoma. *Gastroenterology*. 2009;136:1741-9.
68. Gidekel Friedlander SY, Chu GC, Snyder EL, *et al*. Context-dependent transformation of adult pancreatic cells by oncogenic K-Ras. *Cancer Cell*. 2009;16:379-89.
69. Apelqvist A, Li H, Sommer L, *et al*. Notch signalling controls pancreatic cell differentiation. *Nature*. 1999;400:877-81.
70. Habener JF, Kemp DM, Thomas MK. Minireview: transcriptional regulation in pancreatic development. *Endocrinology*. 2005;146:1025-34.
71. Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell*. 2009;136:823-37.
72. Luo J, Emanuele MJ, Li D, *et al*. A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the Ras oncogene. *Cell*. 2009;137:835-48.
73. Scholl C, Frohling S, Dunn IF, *et al*. Synthetic lethal interaction between oncogenic KRAS dependency and STK33 suppression in human cancer cells. *Cell*. 2009;137:821-34.
74. Singh A, Greninger P, Rhodes D, *et al*. A gene expression signature associated with "K-Ras addiction" reveals regulators of EMT and tumor cell survival. *Cancer Cell*. 2009;15:489-500.
75. Thomas RM, Toney K, Fenoglio-Preiser C, *et al*. The RON receptor tyrosine kinase mediates oncogenic phenotypes in pancreatic cancer cells and is increasingly expressed during pancreatic cancer progression. *Cancer Res*. 2007;67:6075-82.
76. Fisher GH, Wellen SL, Klimstra D, *et al*. Induction and apoptotic regression of lung adenocarcinomas by regulation of a K-Ras transgene in the presence and absence of tumor suppressor genes. *Genes Dev*. 2001;15:3249-62.
77. Engelman JA, Chen L, Tan X, *et al*. Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med*. 2008;14:1351-6.
78. Engelman JA, Janne PA. Mechanisms of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. *Clin Cancer Res*. 2008;14:2895-9.
79. Heyer J, Yang K, Lipkin M, Edelmann W, Kuchlerlapati R. Mouse models for colorectal cancer. *Oncogene*. 1999;18:5325-33.
80. Hutchinson JN, Muller WJ. Transgenic mouse models of human breast cancer. *Oncogene*. 2000;19:6130-7.
81. Klinakis A, Szabolcs M, Chen G, Xuan S, Hibshoosh H, Efstratiadis A. Igf1r as a therapeutic target in a mouse model of basal-like breast cancer. *Proc Natl Acad Sci U S A*. 2009;106:2359-64.
82. Zender L, Spector MS, Xue W, *et al*. Identification and validation of oncogenes in liver cancer using an integrative oncogenomic approach. *Cell*. 2006;125:1253-67.
83. Chan IT, Gilliland DG. Oncogenic K-ras in mouse models of myeloproliferative disease and acute myeloid leukemia. *Cell Cycle*. 2004;3:536-7.
84. Karapetis CS, Khambata-Ford S, Jonker DJ, *et al*. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med*. 2008;359:1757-65.
85. Garcia-Saenz JA, Sastre J, Diaz-Rubio Garcia E. Biomarkers and anti-EGFR therapies for KRAS wild-type metastatic colorectal cancer. *Clin Transl Oncol*. 2009;11:737-47.
86. Moore MJ, Goldstein D, Hamm J, *et al*. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol*. 2007;25:1960-6.
87. Welch SA, Moore MJ. Erlotinib: success of a molecularly targeted agent for the treatment of advanced pancreatic cancer. *Future Oncol*. 2007;3:247-54.
88. Edelman MJ, Sekine I, Tamura T, Saijo N. Geographic variation in the second-line treatment of non-small cell lung cancer. *Semin Oncol*. 2006;33:S39-44.
89. Kobayashi S, Boggon TJ, Dayaram T, *et al*. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med*. 2005;352:786-92.
90. Pao W, Miller VA, Politi KA, *et al*. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med*. 2005;2:e73.
91. Yauch RL, Januario T, Eberhard DA, *et al*. Epithelial versus mesenchymal phenotype determines in vitro sensitivity and predicts clinical activity of erlotinib in lung cancer patients. *Clin Cancer Res*. 2005;11:8686-98.
92. Hertz DL, McLeod HL, Hoskins JM. Pharmacogenetics of breast cancer therapies. *Breast*. 2009;18 Suppl 3:S59-63.
93. Wong KK. Searching for a magic bullet in NSCLC: the role of epidermal growth factor receptor mutations and tyrosine kinase inhibitors. *Lung Cancer*. 2008;60 Suppl 2:S10-8.
94. Sos ML, Michel K, Zander T, *et al*. Predicting drug susceptibility of non-small cell lung cancers based on genetic lesions. *J Clin Invest*. 2009;119:1727-40.
95. Singh M, Lima A, Molina R, *et al*. Assessing therapeutic responses in Kras mutant cancers using genetically engineered mouse models. *Nat Biotechnol*. 2010;28:585-93.
96. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. *Nat Med*. 2004;10:789-99.
97. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000;100:57-70.
98. Yuan TL, Cantley LC. PI3K pathway alterations in cancer: variations on a theme. *Oncogene*. 2008;27:5497-510.
99. Resor L, Bowen TJ, Wynshaw-Boris A. Unraveling human cancer in the mouse: recent refinements to modeling and analysis. *Hum Mol Genet*. 2001;10:669-75.
100. Nambiar PR, Nakanishi M, Gupta R, *et al*. Genetic signatures of high- and low-risk aberrant crypt foci in a mouse model of sporadic colon cancer. *Cancer Res*. 2004;64:6394-401.
101. Glinsky GV, Berezovska O, Glinskii AB. Microarray analysis identifies a death-from-cancer signature predicting therapy failure in patients with multiple types of cancer. *J Clin Invest*. 2005;115:1503-21.
102. O'Hagan RC, Brennan CW, Strahs A, *et al*. Array comparative genome hybridization for tumor classification and gene discovery in mouse models of malignant melanoma. *Cancer Res*. 2003;63:5352-6.
103. Hackett CS, Hodgson JG, Law ME, *et al*. Genome-wide array CGH analysis of murine neuroblastoma reveals distinct genomic aberrations which parallel those in human tumors. *Cancer Res*. 2003;63:5266-73.
104. Kabbarah O, Chin L. Advances in malignant melanoma: genetic insights from mouse and man. *Front Biosci*. 2006;11:928-42.
105. Watters JW, Cheng C, Majumder PK, *et al*. De novo discovery of a gamma-secretase inhibitor response signature using a novel in vivo breast tumor model. *Cancer Res*. 2009;69:8949-57.
106. Jimeno A, Feldmann G, Suarez-Gauthier A, *et al*. A direct pancreatic cancer xenograft model as a platform for cancer stem cell therapeutic development. *Mol Cancer Ther*. 2009;8:310-4.