The Evolution of Mouse Models of Cancer: Past, Present, and Future

Cory Abate-Shen[1] and Katerina Politi[2]

[1]Departments of Molecular Pharmacology and Therapeutics, Urology, Pathology and Cell Biology, Medicine, and Systems Biology, Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center, New York, New York 10032, USA

[2]Departments of Pathology and Internal Medicine (Section of Medical Oncology) and Yale Cancer Center, Yale School of Medicine, New Haven, Connecticut 06405, USA

Correspondence: cabateshen@columbia.edu; katerina.politi@yale.edu

In the nearly 50 years since the original models of cancer first hit the stage, mouse models have become a major contributor to virtually all aspects of cancer research, and these have evolved well beyond simple transgenic or xenograft models to encompass a wide range of more complex models. As the sophistication of mouse models has increased, an explosion of new technologies has expanded the potential to both further develop and apply these models to address major challenges in cancer research. In the current era, cancer modeling has expanded to include nongermline genetically engineered mouse models (GEMMs), patient-derived models, organoids, and adaptations of the models better suited for cancer immunology research. New technologies that have transformed the field include the application of CRISPR-Cas9-mediated genome editing, in vivo imaging, and single-cell analysis to cancer modeling. Here, we provide a historical perspective on the evolution of mouse models of cancer, focusing on how far we have come in a relatively short time and how new technologies will shape the future development of mouse models of cancer.

As we complete a new collection of reviews on mouse cancer models, it is worth reflecting on the remarkable progress the field has made since we summarized the state of the art a decade ago in *Mouse Models of Cancer: A Laboratory Manual* (Abate-Shen et al. 2013). The new collection highlights many approaches and new technologies that were either emerging or not yet conceived of in that edition, which focused on major advances since the earliest days of modeling cancer in mice (Fig. 1).

In those early days (~1980s), the "models" referred primarily to transgenic mice expressing oncogenes in a tissue-specific manner, resulting in tumors in these tissues. Some of the earliest of these genetically engineered mouse models (GEMMs) expressed viral oncogenes like SV40 large T antigen or polyoma middle T antigen (PymT) in prostate or breast or other tissues, leading to cancer development at those sites (Stewart et al. 1984; Hanahan 1985; Guy et al. 1992; Maroulakou et al. 1994; Greenberg et al. 1995; Hanahan et al. 2007). However, there was initially little consideration given as to whether the tumors were arising in the "right" cell types or even whether the choice of a pleiotropic viral

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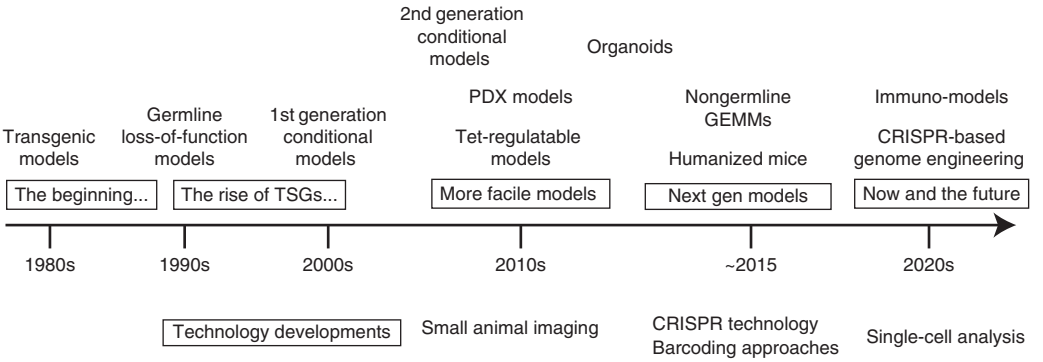

Figure 1. Evolution of mouse modeling through the decades.

oncogene was appropriate, and often the histological phenotypes did not resemble their human counterparts. Nevertheless, these models established the feasibility of modeling cancer in mice, a monumental advance at the time. Remarkably, some of these early models, particularly breast cancer models like the MMTV-PymT model, continue to be informative to this day (Attalla et al. 2021).

Early mouse cancer modeling provided us with fundamental insights into the mechanisms of tumor progression. This is exemplified by the RIP-TAG model (there are many other examples too), in which SV40T antigen was expressed under the control of the rat insulin promoter, leading to islet cell carcinomas (Hanahan 1985). In this model, the pancreatic tumors develop through several stages with normal islets becoming hyperplastic and some of these then undergoing an angiogenic switch leading eventually to frank islet cell carcinomas. However, not all islets that express SV40 T antigen give rise to islet cell carcinomas, which highlighted how additional mechanisms beyond the presence of the driver oncogene are required for tumor progression to occur. Another fundamental observation from this model came from the finding that hyperplastic islets can stimulate the growth of new blood vessels, thus engaging the microenvironment in the process of tumor progression. These early studies of tumor progression highlighted the importance of the tumor microenvironment (TME) and nonmutational mechanisms in tumor progression, and were among the earliest studies to emphasize the role of the immune microenvironment, which have served as the foundation for current studies of tumor progression and metastasis as described in McAndrews et al. (2024), Mahmoud and Ganesh (2023), and Bosenberg (2024).

Next, the field shifted toward tumor suppressor genes, which were emerging as critical determinants of cancer progression, such that loss-of-function models based on the inactivation of tumor suppressors would complement the gain-of-function mouse models based on the expression of oncogenes. Among the earliest models in this era were those based on germline loss of function of *Trp53* and *Retinoblastoma* (*Rb1*) (Clarke et al. 1992, 1993; Donehower et al. 1992; Jacks et al. 1992, 1994; Lee et al. 1992). In the case of *Trp53* loss, most homozygous mutant mice were viable, indicating the somewhat surprising observation that p53 is not essential for embryonic development, while both the heterozygous and the homozygous mutant mice developed cancer phenotypes. However, the spectrum of tumor phenotypes bore little resemblance to the tumors arising following *TP53* dysregulation in human cancers, since these were mostly lymphomas and sarcomas rather than epithelial cancers (Donehower et al. 1992; Clarke et al. 1993; Jacks et al. 1994). It was later shown that age-associated epithelial cancers do not arise in these mice because they are missing a key "mutational" mechanism driving genome change needed to fuel epithelial carcinogenesis, namely, age-associated telomere erosion

and dysfunction against the background of deactivated DNA damage signaling (Artandi and DePinho 2000; Chakravarti et al. 2021).

In the case of *Rb1*, homozygous mutant mice displayed embryonic lethality, indicating that it was essential for normal development, and while the heterozygotes developed cancer phenotypes, surprisingly, they did not develop retinoblastoma, which was the tumor type through which the *RB* gene was first identified. In fact, it was not until years later that investigators showed that only upon knockout of the RB family member p107, in conjunction with Rb1 loss, did retinoblastomas develop in mice in contrast to humans (Robanus-Maandag et al. 1998; Zhang et al. 2004). This became a recurrent theme; for example, germline loss of Phosphatase and TENsin (*Pten*) in mice resulted in embryonic lethality, and while the heterozygous mutant mice developed cancer, the tumor spectrum was only partially overlapping with that for *PTEN* loss in human cancer (Li et al. 1997; Di Cristofano et al. 1998; Podsypanina et al. 1999). Similarly, germline loss of the breast cancer–associated genes, *Brca1* and *Brca2*, resulted in embryonic lethality while the heterozygotes did not develop breast cancer (Evers and Jonkers 2006). Overall, these findings revealed that many tumor suppressor genes, with the surprising exception of *Trp53*, are essential for normal development and differentiation, and their loss results in cancer predisposition but with cancer phenotypes that are not typical of their human counterparts.

To circumvent the consequences of germline loss of function of tumor suppressor genes and to enable investigations of their roles in specific tissue types, in the early 2000s the field moved away from germline alleles and toward the development of conditional alleles allowing for gene recombination in more limited contexts, most often in tissue-specific contexts (Deng 2014). Now, most GEMMs use conditional knockout (loss-of-function) and knockin (gain-of-function) alleles, most often based on Cre–LoxP recombination, in which a specific sequence of DNA flanked by direct repeats of loxP sites can be deleted by a site-specific recombinase enzyme called Cre recombinase. The loxP sites can be engineered to flank essential exons thereby resulting in excision of those exons following Cre recombination, and inactivation of the gene (e.g., conditional knockout allele). All of the classic tumor suppressors, namely, *Trp53*, *Rb1*, *Pten*, *Brca2*, etc., are now represented by multiple distinct versions of conditional knockout alleles (Deng 2014).

Alternatively, conditional knockin alleles can be engineered with loxP sites flanking a stop sequence containing multiple stop codons, such that Cre recombination will result in the removal of the stop sequence and expression of the downstream gene. This technology has enabled the controlled expression of oncogenes in tissue-specific contexts, while circumventing issues related to transgenic alleles, including random transgene integration sites and the complexities of complex crosses. Indeed, the archetypal allele—the conditionally activatable K-Ras (G12D) ($Kras^{LSL\text{-}G12D}$) model that was developed nearly 20 years ago—is still among the most widely used and important mouse alleles for many cancer types (Jackson et al. 2001; Tuveson et al. 2004). Knockin conditional gene recombination has also enabled the expression of reporter alleles for lineage tracing, allowing for spatial and temporal investigations of progression and metastasis in vivo as well as for imaging as discussed in Pitarresi and Stanger (2023), Damoci et al. (2024), Rajbhandari et al. (2024), and Reeves and Balmain (2024).

In addition to conditional alleles engineered to have loxP sites, implicit is the need for tissue-specific promoters to direct the expression of Cre recombinase to achieve conditional gene recombination in those tissue-specific contexts. This has most often been achieved by expressing Cre under the control of a tissue-specific promoter via transgenic or "knockin" alleles, with the caveat that such promoter constructs can lead to misleading interpretations since expression may not be as specific as originally envisioned. Alternatively, delivery of Cre using viral vectors (e.g., adenovirus, lentivirus) has also been a valuable approach in some tissues, including the lung and bladder, thereby overcoming the need for an additional mouse allele and simplifying the complexity of the crosses to generate the relevant mouse line (Ferone et al. 2020).

Further improvements in Cre recombination technology included the introduction of regulatable Cre recombinase, such that gene recombination can be controlled both spatially and temporally. The most common being Cre^{ERT2} alleles, in which Cre recombinase (Cre) is fused to a mutant estrogen ligand–binding domain (ERT2) that does not respond to endogenous estradiols but can be activated by tamoxifen (Feil et al. 1996; Indra et al. 1999; Tian and Zhou 2021). These inducible alleles are particularly advantageous for lineage-tracing studies using, for example, fluorescently activatable reporter alleles (Blanpain 2013).

In recent years, a complementary approach using FLP/FRT-mediated recombination in which FLP recombinase acts on its target (FRT) has been introduced (Deng 2014). A second approach is the Dre–rox system, which uses a site-specific recombinase, Dre, and its target rox sites, which are distinct in their specificity from Cre–loxP (Anastassiadis et al. 2009). The use of Dre–rox has been augmented by the recent development of a series of Dre–rox alleles (Han et al. 2021). A major application of these Frt and Dre systems is their ability to allow for intersectional recombination, which allows for specific gene deletion or activation in a cell type defined by the coactivation of two different promoters controlling Cre and FLP/Dre activity (Awatramani et al. 2003). In addition, when combined with Cre, these site-specific recombination systems allow for temporal control of recombination of different alleles, which can provide information on the role of different genes at distinct time points in tumor progression as well as the effects of restoration of expression of specific tumor suppressor genes (Feldser et al. 2010). However, this also introduces an increasing complexity of allelic combinations since this requires alleles flanked by FRT or DRE rather than loxP sites.

Another way to regulate inducible tissue-specific expression is with a tetracycline-regulated system, which has been particularly effective for mouse models of breast and lung cancer among others (Yeh et al. 2014). A major advantage of tet-regulated systems is that, in addition to inducing expression of a gene of interest at a defined time point by addition (for a Tet-ON system) or removal (for Tet-OFF) of the tetracycline analog doxycycline, it is fully reversible by withdrawal (or addition) of doxycycline. This approach has allowed investigators to test the role of several oncogenic variants in tumor maintenance (Chin et al. 1999; Fisher et al. 2001; Politi et al. 2006) and has also been valuable to establish the difference between inactivating an oncogene by losing its expression compared to inhibiting its activity (Kwong et al. 2012). Tetracycline regulation has also been leveraged to silence gene expression using short hairpin RNAs (shRNAs). In one such approach, shRNAs under the control of a tetracycline-inducible promoter, are targeted to the *Col1a1* locus allowing for the presence of a single copy of the tet-regulated shRNA in embryonic stem (ES) cells (that can also contain the reverse tetracycline transactivator under a ubiquitous or tissue-specific promoter). Proof-of-principle studies demonstrated that this approach could be used to efficiently knock down the expression of tumor suppressor genes like *Apc* leading to tumorigenesis in vivo (Premsrirut et al. 2011).

One opportunity that emerges from developing tissue-specific approaches to regulate gene recombination or expression is that this can be directed to specific cell types within the tissue, such as subtypes of epithelial cells, which has provided insights into cells of origin of different types of cancer as well as further refinement of mouse models that can be used to study cancer. For example, in the prostate, a cell-type-specific Cre allele was used to demonstrate that luminal cells are a cell of origin of prostate cancer (Wang et al. 2009). Similarly, in the lung, the sophisticated use of conditional alleles has established a key role for alveolar type 2 (AT2) cells in giving rise to lung adenocarcinomas (Ferone et al. 2020). Furthermore, in the lung as well as in the bladder, the ease of delivering viral vectors with Cre recombinase under the control of cell-type-specific promoters has enabled the definition of cells of origin for cancer subtypes (Ferone et al. 2020; Park et al. 2021). In studies of the cell of origin of small-cell lung cancer (SCLC), delivery of Cre to AT2 cells, neuroendocrine cells, or Clara cells to the lungs of mice harboring floxed alleles of *Trp53* and *Rb* (inactivation of which leads to SCLC), revealed that neuroendocrine

cells and Clara cells were the most and least likely to give rise to SCLC, respectively (Sutherland et al. 2011). Recently, such approaches have provided insights into the mechanisms that underlie histological transformation that can be observed at the time of resistance to targeted agents in prostate and lung cancer (Zou et al. 2017; Chan et al. 2022; Gardner et al. 2024).

The culmination of these efforts and refinements over several decades has resulted in a plethora of GEMMs that represent the most common cancer types, which capture a range of relevant phenotypes for their respective cancer types, from preinvasive/early invasive lesions to highly metastatic disease, and that are well representative of the biological and molecular features of their corresponding human counterparts. As these models have evolved, their analyses have provided information on the hallmarks of cancer, helped to understand basic mechanisms of disease initiation and progression, provided insights into strategies for prevention, provided resources to evaluate new therapeutic opportunities, and helped to understand therapeutic resistance. Articles in this new collection highlight the seminal contributions of GEMMs to our understanding of these processes, ranging from studies of the cell cycle, and cancer metabolism to tumor evolution and therapeutics (Gultekin et al. 2023; Hammond and Sage 2023; Reeves and Balmain 2024; Vaishnavi et al. 2024).

A particularly important application of these GEMMs has been their use for preclinical investigations, fueled by the advent of targeted therapies and immunotherapies that can be investigated in models. Indeed, a combination of factors has stimulated studies of therapeutic agents in preclinical models in recent years, including the availability of GEMMs that more accurately reflect the genotypes of human tumors, complemented by novel imaging approaches to visualize tumors in vivo (Damoci et al. 2024; Rajbhandari et al. 2024) and the increased establishment of high-fidelity patient-derived xenograft (PDX) and patient-derived organoid (PDO) models (Cocco and de Stanchina 2023; Love and Karthaus 2023). For example, the availability of a comprehensive series of GEMMs of prostate cancer enabled the establishment of a personalized systems biology approach to identify drug candidates for individual patient tumors. In particular, the comparison of individual GEMM tumors with individual human patient tumors combined with interrogation of drug perturbation studies led to the prediction of drugs that enhanced the efficacy of clinically relevant drugs, namely, the PD1 inhibitor, nivolumab, and the AR-inhibitor, enzalutamide (Vasciaveo et al. 2023). Additionally, in a recent study using breast cancer PDXs and PDX-derived organoids, investigators uncovered a therapeutic vulnerability of a treatment-refractory tumor and were able to treat the patient with the corresponding agent and observed a response (Guillen et al. 2022). Furthermore, studies in GEMMs of lung adenocarcinoma have provided information on the sensitivity of different oncogenic subsets of the disease to targeted therapies and have shed light on mechanisms of resistance to these agents as elaborated in Vaishnavi et al. (2024). These examples highlight how studies in modeling cancer in mice can provide important actionable insights for patients revealing their utility in the era of precision medicine.

The advent of immunotherapy has represented a new opportunity and challenge for the field as described in Bosenberg (2024). In recent years, investigators have grappled with identifying optimal models to test and study immunotherapeutic strategies. At first glance, GEMMs should appear optimal as tumors develop progressively over time in the native TME within an immunocompetent setting. However, tumors that develop in GEMMs generally have a lower tumor mutational burden (TMB) compared to their human counterparts (George et al. 2015; McFadden et al. 2016). Since TMB is an important factor contributing to sensitivity to T-cell-directed therapies like PD(L)-1 axis blockade, many GEMM tumors are insensitive to these agents. To circumvent this issue, new GEMMs and GEMM-derived cell lines have been developed for cancer immunology research in which the tumors can more easily be recognized by the immune system either through expression of a model T-cell antigen or due to an increased tumor mutation burden (Wang et al. 2017; Damo

et al. 2021; Germano et al. 2021; Westcott et al. 2023). The situation is even more complex for PDX models, which are typically propagated in immunodeficient mice. Humanized mice that support the growth of human immune cells and in vitro coculture approaches have been developed that enable studies of human tumor models and immunotherapies (Politi 2020).

However, despite the many advances in developing mouse models of cancer, the recurrent central question is whether studying cancer in GEMMs can really inform studies of human cancer in a clinically actionable way. The legitimate basis for concern is that mice are less likely to develop neoplasias spontaneously, which is not the case for other species, such as rats, dogs, and even pigs. Nonetheless, mice are the preferred species for cancer modeling because of the feasibility of maintaining large colonies, and the relative ease of manipulating the genome, although it is becoming increasingly feasible to manipulate the genome of rats (Lutz et al. 2022). Although the underlying reasons that mice do not develop cancer spontaneously are unknown, a major difference in mice compared with humans (and other species) is telomere length. Indeed, mice that were genetically engineered to have eroded telomeres over several generations develop tumors with greater incidence and aggressiveness (Lee et al. 1998; Rudolph et al. 1999; Artandi and DePinho 2000).

This has not diminished the concerns of the naysayers who wonder whether by studying cancer in mice, we are actually making inroads into human cancer, or whether we are simply "curing cancer in mice." Unfortunately, much of the concern stems from a failure to distinguish between autochthonous models and xenograft models. Autochthonous models generally refer to GEMMs in which tumors arise from normal cells over time and in the context of the native TME, which we now appreciate to have a key role for all aspects of cancer progression, metastasis, drug response, and resistance. In contrast, nonautochthonous xenograft models, as distinguished from syngeneic allograft models, refer to tumors propagated in immunodeficient hosts. These, therefore, lack a native TME, and often are implanted subcutaneously so they lack a native tissue microenvironment. In the earliest generation of these xenograft models—and still widely used—the tumors are generated from cancer cell lines that have been in culture for many years.

In some cases, studies in such xenograft models have provided important insights into targeted therapies, such as for example studies in the LNCaP prostate cancer cells, which were important for the development of enzalutamide (Abate-Shen and Nunes de Almeida 2022). However, in general, these models have proven to be unreliable for informing on their human counterparts, and particularly ineffective for providing reliable insights into drug efficacy, which has generally given mouse models a "bad reputation." Xenograft models have now improved in many respects, as is the subject of the contribution by Cocco and de Stanchina (2023). Many PDX models are now based on patient-derived tumors or organoids, rather than cultured cell lines, and are now often propagated orthotopically in the relevant tissue microenvironments, even in some cases in which both the stroma and epithelial components are of human origin (Kuperwasser et al. 2004). These more sophisticated models have been leveraged to inform on drug response, as discussed above, as well as to study tumorigenic processes including metastasis and dormancy as described in Mahmoud and Ganesh (2023). As described above, a major advance is the application of humanized mouse hosts; however, these are not generally accessible because of their high cost.

In terms of patient-derived models, the development of organoid models, which are three-dimensional avatars of tumors, has made a tremendous impact and those are the subject of Love and Karthaus (2023). In pancreatic cancer, for example, a cancer type that is particularly dependent on tumor–stroma interactions, studies of organoid models have made major inroads for informing on the tumor and microenvironment interactions that drive disease progression (Boj et al. 2015). Additionally, these models have been used to study determinants of chemotherapy sensitivity in pancreatic ductal adenocarcinoma and to identify alternative approaches to treat chemorefractory tumors (Tiriac et al. 2018).

In colorectal cancer, a comparison of responses to anticancer agents ex vivo in organoids and PDO-based orthotopic mouse tumor xenograft models with the responses of the patients in clinical trials showed they were strongly concordant (Vlachogiannis et al. 2018). Additionally, a comprehensive analysis of metachronous organoid lines from bladder cancer revealed changes in organoid drug response following patient treatment (Lee et al. 2018).

The most significant advances in mouse modeling over the past decade have stemmed from a surge of new technologies. These innovations have markedly enhanced our capacity to develop more refined models efficiently, allowing us to delve deeper into the cellular, molecular, and systemic processes involved in disease progression. Among these breakthroughs is the emergence of nongermline GEMMs, a concept already on the horizon at the time we last considered the state of the field (Heyer et al. 2010), expounded upon in Murphy and Ruscetti (2024). Nongermline GEMMs offer a distinct advantage by enabling the introduction or induction of genetic alterations into specific cells within a tissue, mirroring scenarios observed in humans more closely. These models are playing an increasingly pivotal role in elucidating the interplay of various genetic drivers across different cancer types.

The development of nongermline GEMMs has coincided with the explosion of CRISPR-based gene-editing techniques that allow the rapid introduction of genomic alterations, including point mutations, into somatic tissues in mice and that have transformed the field as described in Sánchez Rivera and Dow (2023) and Tang et al. (2023). CRISPR-Cas9 genome editing facilitates the modeling of complex tumor genotypes, the identification of genes that modulate therapeutic sensitivity, and can reveal new vulnerabilities of tumors. Looking ahead, CRISPR lays the groundwork for technologies that enable the study of hard-to-study cancer biological processes such as tumor evolution, using CRISPR-based molecular recording.

The advent of single-cell technologies has also profoundly impacted cancer biology, including studies involving mouse models of cancer. These cutting-edge tools enable investigations into the properties of individual cancer cells, including their cell state and features of the TME, offering unprecedented insights into tumor heterogeneity and biology at the transcriptomic, genetic, and epigenetic levels (LaFave et al. 2020; Marjanovic et al. 2020; Baysoy et al. 2023). For instance, single-cell analysis of pancreatic ductal adenocarcinoma in mice revealed distinct subsets of cancer-associated fibroblasts, including an antigen-presenting subset capable of modulating the immune system (Elyada et al. 2019). Similarly, single-cell transcriptomic analysis uncovered a role for p53 in the differentiation of alveolar type 1 cells in the lungs (Kaiser et al. 2023). Although single-cell analysis at the protein level presents technical challenges, advancements in this area are imminent. Nascent spatial imaging approaches hold promise in significantly contributing to our understanding of tumorigenesis in mouse models (see, e.g., Zhao et al. 2022).

Ultimately, the lingering question remains: are we truly advancing our understanding of human cancer, or are merely "curing" cancer in mice? Over the next decade, armed with increasingly sophisticated models, powerful technologies, and analytical methods, we anticipate substantial examples of critical insights gained from studying mice. These insights will undoubtedly impact our knowledge of human cancers and, most importantly, contribute to improving patient outcomes and their care—our shared mission.

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