Mouse Modeling in Oncologic Preclinical and Translational Research

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With the identification of molecular pathways involved in tumorigenesis, novel models of human cancer have been developed to further our understanding, evaluate potential therapies, and ultimately impact on patient outcome. The mouse has provided an excellent platform for modeling cancer in a mammalian system. This has traditionally been accomplished with the use of xenograft models incorporating primary or genetically altered cell lines derived from primary and metastatic tumors. As our ability to manipulate the mouse genome expanded, it has become possible to evaluate specific molecular pathways involved in carcinogenesis. Currently, mouse modeling of human cancer is possible through the expression of oncogenes, specific genetic mutations, or the inactivation of tumor suppressor genes, and these experiments have begun to provide us with an understanding of the molecular pathways involved in tumor initiation and progression. Additionally, these mouse models serve as an excellent system to evaluate the efficacy of currently developed molecular targeted therapies and identify new potential targets for future therapies. To keep this review concise, we will focus on the generation and study of genetically altered mouse models for human acute promyelocytic leukemia (APL) and prostate cancer, two diseases that we currently study in our lab. Through this, we will show the usefulness of mouse modeling in oncologic translational research.

From Human to Mice: Modeling Tumorigenesis

Through advances in the molecular analysis of human tumors, several well-defined and common molecular pathways have been found to be dysregulated in malignancies (1–3). As knowledge was increasing about the identification of specific genetic alterations in cancers, our ability to manipulate the mouse genome was expanding. Broadly, genetically engineered mouse models of cancer encompass mice overexpressing a transgene (oncogene or point mutation), knock-in models of genetic point mutations, as well as complete knock-out and conditional knock-out models using the cre-lox system (4, 5). Taking advantage of the knowledge we have gained by examining genetic alterations in human malignancies, a number of studies have been done to evaluate the effect of these genetic alterations in mouse models (Fig. 1). These experiments have helped define the cellular responses to specific genetic alterations and their effect on the organismal phenotype. From these studies we have learned the mechanisms that confer selective advantage to tumor cells, such as disruption of cell cycle regulation and inhibition of apoptotic pathways. Whereas a number of mouse models have been generated and contributed greatly to the study of carcinogenesis, we have selected a few examples of mouse models of APL and prostate cancer to show their usefulness in establishing the molecular pathways involved in human tumorigenesis and their usefulness in translational research.

Modeling APL in the Mouse

APL accounts for >10% of all acute myelogenous leukemias and is characterized by distinct and unique features (6, 7). APL is associated with the accumulation in the bone marrow of tumor cells with promyelocytic features and the invariable association with specific reciprocal chromosomal translocations involving the retinoic acid receptor α (RARα) gene on chromosome 17 (6, 7). In the vast majority of cases (>98%), the RARα gene fuses to the promyelocytic leukemia gene, PML. In a small subset of cases, RARα has been found to fuse to several other genes that have recently been identified (PLZF, NPM, NUMA, or STAT5b) leading to the generation of X-RARα and RARα-X fusion proteins (8–11). The involvement of RARα in the pathogenesis of APL made this leukemia a straightforward example of aberrant transcription in tumorigenesis. The RARs belong to the superfamily of nuclear hormone receptors, which act as transcription factors. These molecules are involved in fundamental biological processes, such as development and...
differentiation. APL translocations result in the generation of X-RARα and RARα-X fusion genes and their coexpression in the leukemic blasts, resulting in the interference of both RARα and X-gene pathways. To evaluate the role of these fusion proteins in leukemogenesis, a number of groups have attempted to generate mouse models that express these fusion proteins in the myeloid compartment of the bone marrow under the control of hemopoietic tissue-specific promoters (12–15). Through these efforts, it has been shown that the expression of PML-RARα, PLZF-RARα, NPM-RARα, and NUMA-RARα results in leukemic development in mice following a long latency and with variable penetrance. Additionally, these transgenic mice display distinct cytomorphic features, depending on the fusion protein expressed. Transgenic mice expressing PML-RARα develop leukemia with classic features of human APL following, however, a long latency with an approximate 10% to 30% penetrance (12, 13). By contrast, transgenic mice expressing the PLZF-RARα fusion protein develop leukemia with chronic myelogenous leukemia–like features with 100% penetrance by 8 months of age (14). Strikingly, however, transgenic mice that coexpress both PLZF-RARα and the reciprocal translocation product RARα-PLZF develop leukemia with an APL phenotype. Modeling APL through the overexpression of RARα fusion proteins has provided concrete proof for the role of these proteins in leukemogenesis. Additionally, the specific translocation products contribute distinct molecular and morphologic features to the developing leukemia, which may play a role in sensitivity to therapy, as we will discuss in the following section. Therefore, mouse models serve as an excellent platform for the molecular analysis of pathways involved in the initiation and progression of malignancies as well as the evaluation of therapeutic agents.

Modeling Prostate Cancer in the Mouse through the Loss of PTEN

The tumor suppressor gene phosphatase and tensin homologue (PTEN) is mutated in a variety of human malignancies at a frequency roughly equal to that of p53 (16). PTEN, a lipid phosphatase, is a negative regulator of the phosphatidylinositol 3-kinase/AKT pathway, which is frequently activated in a variety of malignancies. It has previously been reported that ~70% of primary prostate cancers show loss of at least one allele of PTEN, whereas homozygous inactivation of PTEN is generally associated with advanced cancer and metastases (17). In an effort to define the role of PTEN loss in prostate tumorigenesis, a series of PTEN loss mouse models (PTEN+/−, PTEN hypomorphic, and PTEN conditional knock-out) have been generated. This has allowed us to determine the critical importance of subtle variations in the level of PTEN on prostate tumorigenesis. These analyses allowed us to reach a number of important conclusions: (i) Loss of PTEN is critical for prostate cancer initiation (18, 19). (ii) The level of PTEN expression is inversely associated with prostate tumorigenesis. For instance, loss of one allele of PTEN is associated with the development of high-grade prostatic intraepithelial neoplasia.
studies have shown that loss of PTEN in vivo known to influence cancer susceptibility. Taken together, these the different genetic background strain of the mice, which is our PTEN loss mouse model. This may be, perhaps, secondary to the different genetic background strain of the mice, which is known to influence cancer susceptibility. Taken together, these studies have shown that loss of PTEN in vivo results in activation of the AKT pathway and susceptibility to tumorigenesis, which is closely related to the degree of PTEN insufficiency. (iii) Additionally, loss of PTEN has been shown to cooperate with the loss of other tumor suppressors frequently found inactivated in prostate cancer (p27 and p53) to accelerate tumorigenesis (21, 22). Mice nullizygous for PTEN and p53 in the prostate (prostate conditional knock-out) develop a locally aggressive, lethal prostate cancer, with a complete penetrance. Importantly, through this research, we have learned that PTEN and p53 cooperate in a specific manner to accelerate tumorigenesis. Complete loss of PTEN triggers, in fact, a fail-safe cellular response to oncogenic stress, known as cellular senescence, which occurs through a p53-dependent mechanism (Fig. 2; ref. 22). Cellular senescence describes an irreversible cell cycle arrest phenotype, which is triggered by cellular stress such as the activation of oncogenes. Thus, complete loss of PTEN in the prostate results in an indolent prostate cancer, due to the fact that PTEN inactivation in the prostate triggers in vivo a p53 cellular senescence response, thereby limiting the progression of cancer. Therefore, concomitant or sequential prostatic loss of PTEN and p53 results in a dramatic acceleration of prostate tumorigenesis.

As these examples undoubtedly prove, mouse models have allowed us to construct oncogenic pathways, potentially identifying a series of molecular events resulting in tumor initiation and subsequent progression to invasive disease. Understanding the multistep evolution from homeostasis to tumorigenesis to metastasis is paramount for defining therapies that would be effective at various clinical disease states.

### Evaluation and Development of Targeted Therapy in Genetic Mouse Models and Genetically Engineered Primary Cells

With further understanding of the molecular pathways involved in tumorigenesis, there has been an explosion of research in the development and evaluation of potentially effective “targeted therapies,” cancer drugs that target molecular processes thought to be critical of tumor initiation, survival, or progression. Ideally, these malignant processes can be identified before treatment through clinical and pathologic evaluation of the patient and tumor specimen. Arguably, the first form of targeted therapy was the use of antiestrogens in women with breast cancer that overexpressed the estrogen receptor (23, 24). Through immunohistochemical analysis of the tumor specimens, it was possible to identify a population of breast cancer patients who would potentially benefit from antiestrogen therapy. Additionally, through the molecular analysis of APL, it was identified that a reciprocal chromosomal translocation, t(15;17), resulted in a RARα fusion protein and subsequent RARα functional impairment (25, 26). The development of all-trans retinoic acid therapy, targeting the wild-type RAR to induce cellular differentiation, has resulted in high overall response rates, delay in disease progression, and long-term cure rates in select patients with APL (26).

A critical event in the process of therapeutic testing is the preclinical evaluation in the animal model. Thus far, the majority of preclinical trials have been conducted in xenograft mouse models. Whereas xenograft models are straightforward and relatively easy to use, their accuracy in predicting the efficacy of an anticancer agent in patients is questionable (27). This may be secondary to cell line selection, the environment or location of the xenograft transplant, or the lack of de novo tumor development. Mouse models recapitulate human malignancies by de novo tumor development. Mouse models represent the handiest genetic systems to evaluate targeted therapies and perform high-throughput drug screenings. MEF generated from a genetically modified mouse (e.g., a knock-out mouse lacking a tumor suppressor) carries the same genetic alteration, and although it may not be the cell of origin of the modeled phenotype, it allows us to readily evaluate the genetic and molecular processes resulting from the loss of a tumor suppressor or the overexpression of an oncogene. These cells also allow us to rapidly evaluate potential targeted therapies, the ability of a drug to hit its molecular target and its mechanism of action in various genetic backgrounds, as well as to identify new targets of interest (Fig. 3). A caveat exists with the use of MEF in that these cells, while providing excellent genetic and molecular evaluation, are frequently not the cells of origin for the majority of malignancies evaluated and, therefore, cellular specific processes may vary. Therefore, whereas MEF allows for the screening of molecular events and therapeutic strategies, experiments must be confirmed in the origin of the malignant cells in preclinical trials with mouse models.

In summary, the mouse model or primary cells derived from these mouse mutants can be effectively used for the development of novel therapeutic modalities. We will provide a few examples of the power of this approach and the impact that it has had on clinical practice in recent years.

### PTEN-AKT Pathway: Examples of Target Inhibition in Genetically Engineered MEF and Mouse Models

Rapamycin, a bacterial macrolide, has been shown to inhibit the mammalian protein mammalian target of rapamycin (mTOR) kinase, which links mitogen stimulation to protein synthesis and cell cycle progression, and seems to be an
attractive anticancer therapeutic target (28). Cancer initiation and progression frequently involves signaling pathways linked to mTOR, such as AKT or extracellular signal–regulated kinase activation, and several clinical trials are currently ongoing with rapamycin and its analogues in breast cancer, prostate cancer, and renal cell carcinoma. Several studies involving MEF have allowed investigators to determine the in vitro effectiveness of rapamycin in various genetic backgrounds, as well as the mechanisms of action in these cells. Rapamycin has been shown to induce a G1 cell cycle arrest in wild-type MEF, which depends on intact p53/p21 genes (29). It was shown that p53- or p21-null MEFs have a diminished ability to undergo G1 cell cycle arrest and instead elicit an apoptotic response to rapamycin treatment (29). Due to the frequency of p53 mutations in human malignancies often coexisting with mutations in genes regulating the mTOR axis, this may in part explain why a molecular targeted therapy, such as rapamycin, confers selectivity to tumor cells and relatively spares normally proliferating cells.

Along the same line, it has been shown that loss of PTEN in both mouse cells and mouse tumors results in an increased activity of mTOR leading to S6 kinase activation, and inhibition of the mTOR pathway with the rapamycin analogue CCI-779 was capable of blocking tumor development (30, 31). Xing and Orsulic further showed, using mouse ovarian epithelial cells expressing the TVA receptor, that specific genetic alterations could be generated in these cells with the RCAS system (32), and furthermore, that rapamycin effectively inhibited the growth of ovarian tumors in vivo, which relied on AKT signaling for proliferation (33). Thus, the use of mouse models allowed us to evaluate the effect of a specific targeted therapy in the face of known genetic alterations.

In addition to the evaluation of various targeted therapies, altering the genetics of the mouse will allow us to identify molecular pathways that may be useful targets for future therapeutic development. As we have previously stated, p53 elicits a cellular senescence response following the loss of PTEN and subsequent activation of AKT. Additionally, loss of p53 in these mouse models accelerates tumorigenesis, confirming the importance of p53 in this oncogenic pathway. Based on these findings, it is attractive to conclude that therapies up-regulating or stabilizing p53 may be effective in PTEN-null tumors early in the process of tumorigenesis before p53 gets lost or mutated. Small-molecule inhibitors that bind to the p53 pocket of murine double minute-2, thereby inhibiting p53 degradation, have been developed (34). This class of p53-enhancing drugs has broad implications as a number of initiation events in cancer development trigger a p53 response, which is frequently overcome by p53 loss. Therefore, stabilization of functional
p53 may lead to cell cycle arrest or cellular death, and this may be particularly effective in the context of PTEN nullizygosity.

With our enhanced knowledge of pathways involved in cancer initiation and progression, a number of molecules seem to be attractive targets of therapy in malignancies with loss of PTEN (Fig. 4). These targets can be easily modeled in knock-out mice to determine their effect on tumorigenesis and whether a screening for potential drugs to inhibit these pathways should be undertaken.

Acute Promyelocytic Leukemia: Evolution of Preclinical Trials

Mouse modeling has been effectively used to evaluate a variety of therapeutic agents for the treatment of APL. From these studies we have gained a substantial knowledge as to which therapies are efficacious and to which degree the molecular basis for the leukemia dictates the response to therapy. As in humans, APL in PML-RARα transgenic mice responds well to retinoic acid therapy, which can induce complete disease remission; hence, this mouse model faithfully recapitulates the human form of the disease, not only in its genetics but also in the responsiveness to therapy (35). Through these preclinical studies, we have also found that leukemic mice harboring the PLZF-RARα fusion protein poorly respond to retinoic acid, and complete disease remission is never attained (35). Therefore, patients with APL who harbor the t(11;17) translocation are not ideal candidates for retinoic acid therapy. Furthermore, additional preclinical studies have shown that the addition of arsenic trioxide to retinoic acid therapy improved survival in PML-RARα transgenic mice (35, 36). This is of importance in that previous investigators concluded that, on the basis of *in vitro* studies, no synergistic effect would result from combination therapy with retinoic acid and arsenic trioxide. Therefore, the efficacy of this combination therapy for APL, which is now in clinical trials, highlights the benefits of mouse modeling. However, no benefit was seen for single or combination therapy in PLZF-RARα transgenic mice, showing the resistant nature of these leukemias. Given that the PML-RARα and PLZF-RARα fusion proteins function as aberrant transcriptional repressors, in part by recruiting nuclear receptor-transcriptional corepressors and histone deacetylases, the efficacy of histone deacetylase inhibitors for the treatment of APL has been evaluated (37). In APL cells harboring the t(15;17) translocation, histone deacetylase inhibitors cause growth inhibition and apoptosis and potentiate retinoic acid–induced differentiation. Furthermore, whereas retinoic acid therapy is not efficacious for the treatment of APL induced by the PLZF-RARα fusion protein, combination

**Fig. 3.** MEFs represent an excellent tool for the evaluation of therapies in various genetic backgrounds, as well as the screening of novel therapies through the use of RNA interference or small-molecule inhibitors. Therapies that result in an efficacious response can then be evaluated in preclinical trials using mouse models that faithfully recapitulate the human form of the disease.
therapy with retinoic acid and a histone deacetylase inhibitor prolonged survival and induced complete remission in transgenic mice without noticeable toxicity (37). On the basis of these results, histone deacetylase inhibitors have subsequently been used in the treatment of human APL (38). From these studies we have gained information about the mechanism of action of these therapeutic agents as well as the molecular setting in which they are most efficacious (Table 1). This will ultimately lead to clinical trials evaluating these therapeutic agents in appropriate patients based on molecular and cytogenic criteria.

From Mice to Humans: The Evolution of Clinical Trials

Mouse models have continued and will continue to provide us with a wealth of information about tumor initiation and progression, potential novel targets and markers of therapy, and the efficacy and tolerance of various anticancer agents. The translation of this data is of paramount importance to improve our ability to prognosticate and ultimately render cancer patients free of disease. With the identification of molecular markers and their role in the process of tumorigenesis, it is currently possible to molecularly stage a cancer patient. Through this, clinicians are capable of identifying patients who would be candidates for enrollment in a targeted therapy clinical trial. This strategy was initially employed for women with breast cancer (23, 24). Following examination of the tumor specimen, clinical trials have shown that women with estrogen receptor/progesterone receptor–positive tumors receive a substantial benefit from adjuvant antiestrogen (tamoxifen) therapy. More recently, gefitinib, a tyrosine kinase inhibitor that has shown efficacy for non–small-cell lung cancers, has emerged as an excellent model of targeted therapy.

Table 1. Response to targeted therapy in mouse models of APL

<table>
<thead>
<tr>
<th>Genetic modification</th>
<th>Response to RA</th>
<th>Response to RA + As$_2$O$_3$</th>
<th>Response to RA + HDAC inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>PML-RAR$_{va}$</td>
<td>CR</td>
<td>CR</td>
<td>CR</td>
</tr>
<tr>
<td>PLZF-RAR$_{va}$</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>NPM-RAR$_{va}$</td>
<td>CR</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviations: RA, retinoic acid; As$_2$O$_3$, arsenic trioxide; HDAC, histone deacetylase; CR, complete response; NR, nonresponsive.
therapy. It has recently been shown that specific mutations in the epidermal growth factor receptor confer sensitivity to gefitinib therapy (39–41). Therefore, molecular characterization of these tumors is essential for dictating appropriate therapy. As we discussed before, APL patients with PML-RARα respond to all-trans retinoic acid therapy. Taken what we have learned from preclinical studies in APL transgenic mice, the combination of retinoic acid and arsenic trioxide therapy is a reasonable form of therapy for patients relapsing following conventional therapy, who harbor the t(15;17) translocation, and more importantly, clinical trials are currently under way evaluating this combination therapy for the primary management of APL (42–44).

It is therefore obvious that future trials evaluating chemo-therapeutic agents for cancer patients should incorporate cytogenetic and molecular markers, as mouse modeling has shown that response to therapy is dictated by the molecular and genetic makeup of the tumor. With the emergence of more data showing the efficacy of targeted therapies in preclinical mouse models, future clinical trials will be designed for cancer patients. In this respect, mouse modeling of APL represents a compelling example for the ideal progression from preclinical evaluation to translational research and has successfully informed clinical trials and altered the management of patients.

References
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