Genetically Engineered Models Have Advantages over Xenografts for Preclinical Studies

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Abstract

Mouse models of human cancer are valuable tools for cancer research. Although xenografts and genetically engineered models (GEMs) possess limitations as well as advantages, each system plays a significant role in preclinical testing. Tumor xenografts are easy to use, relatively inexpensive, and reproducible. The main drawback of xenografts is that the genetics and histology of the tumors are frequently not representative of the respective human tumor and, thus far, these models have not been as predictive of therapeutic success as one would like. By contrast, GEMs are histologically and genetically accurate models of human cancer but have disadvantages of heterogeneity with regard to frequency, latency, and growth. These disadvantages are reminiscent of the variable behavior of actual human tumors. Recently, these shortcomings have been partly overcome with the development of anatomic and molecular in vivo imaging techniques such as magnetic resonance imaging and bioluminescence imaging. These new technologies will hopefully support the use of GEMs in preclinical trials and help determine if trials in GEMs are more predicative than xenografts of human responses. (Cancer Res 2006; 66(7): 3355-9)

Introduction

Advances in molecular biology have significantly increased our understanding of the biology of cancer. However, these discoveries have not yet been fully translated into improved treatments for patients with cancer. One of the factors limiting the translation of knowledge from preclinical studies to the clinic has been the limitations of in vivo cancer models. In this brief review we will discuss the advantages and disadvantages of xenografts and genetically engineered models (GEM) of cancer for preclinical studies. We will focus on GEMs and review some of the current genetic strategies for modeling cancer in the mouse and highlight some of the preclinical studies that have already been undertaken in GEMs. We will also discuss how recent improved imaging technologies in mice promise to make preclinical testing in GEMs more attractive.

Xenografts

Xenografts are human cells or human cell lines grown in an immunodeficient mouse. There are two main sites used for tumor xenografts: ectopic (s.c.) and orthotopic, a term which refers to the native site of the tumor. There are several advantages to s.c. xenografts. The progression of a large number of synchronized, easily observable tumors can be followed, such that initiation of treatment can begin when the tumors are of an optimal size. Furthermore, xenografts have a high degree of predictability and rapidity of tumor formation, which makes them easy to use. Lastly, only a few mice are needed for drug efficacy studies. The primary shortcoming of xenografts is that the human cell lines used have been maintained on a plastic plate for years. These cell lines have been passaged for many generations in culture and, due to selection pressures under these conditions, are not representative of original tumor in its native state. Cells in culture lack the architectural and cellular complexity of in vivo tumors, which include inflammatory cells, vasculature, and other stromal components. Because the genetics and histology of xenografts do not recapitulate the genetics and histology of human tumors, GEMs were developed.

Germ-Line Genetically Engineered Models

The first transgenic mouse tumor model was established by overexpression of viral and cellular oncogenes in specific tissues (1–4). Later, the introduction of gene-targeting technology in mouse embryonic stem cells allowed for the generation not only of oncogene-bearing transgenic mice (gain of function) but also of conventional tumor suppressor gene knockout mice (loss of function). These cancer-prone mouse strains led to the understanding of the role of individual genes and their mutated counterparts in tumorigenesis, as well as the cooperation of individual mutations in tumor development. Cancer is a disease characterized by progressive accumulation of somatic mutations. Such sporadic lesions eventually lead to tumor growth inside a genetically wild-type environment, which often actively contributes to tumor progression. Thus, tumor models that use conventional transgenic or knockout approaches can be used to model familial cancer predisposition syndromes. Additional mutations are acquired in the formation of tumors that develop; sometimes tumors in unintended sites arise before those in the originally intended organ sites. Germ-line mutations of either oncogenes or tumor suppressor genes can lead to embryonic lethality due to unintended effects during development. To overcome these problems, various strategies for conditional gene expression in the mouse have been developed.

Conditional Genetically Engineered Models

Conditional strategies have been developed that allow control of gene expression in both a tissue and temporal specific manner. The first strategy of this nature was based on the use of chemically induced transcription factors, which are responsible for the regulated expression of the target transgene. In this class of approaches, the most widely used is the tetracycline-dependent
regulatory system (5). Another system of conditional loss of function is the conditional/somatic deletion of tumor suppressor genes by site-specific and spontaneous recombination using the Cre- or Flp-mediated recombination. The use of these methods, and newer more complex variants of these strategies, has significantly expanded the array of genetic manipulations that can be carried out in mice to study the pathogenesis of cancer.

Somatic Cell Gene Transfer

Somatic cell gene transfer is another method used for conditional oncogenic expression. Experimentally induced, virally mediated oncogenesis has been used to study cancer for nearly 100 years. The technology differs from germ-line modification strategies in that the viral vectors that induce cancer in experimental animals achieve their goal by transferring genes into a limited number of somatic cells, usually after birth. New advances in these systems now support experimental designs aimed at assessing the effects of mixtures of genetic alterations, identification of the contribution of specific alterations on the histology of tumor cells, and the identification of cell-of-origin for certain cancers. These somatic cell gene transfer strategies are complementary to germ-line modification technologies in unraveling the complexities of cancer biology.

The use of avian retroviral vectors for gene transfer to mice requires that the mouse cells be genetically modified to express the receptor used by the retrovirus. The most commonly used system of this sort uses vectors based on the subgroup A avian leukemia virus, referred to as RCAS vectors (6), and their receptor tv-a (7). Because tv-a is not normally expressed on mammalian cells, the infection with RCAS is extremely low or nonexistent. However, when mammalian cells are engineered to express tv-a, they become highly susceptible to infection by RCAS vectors. The absolute requirement for expression of tv-a by target cells allows the engineering of mice, which limits infectability by RCAS vectors to specific cell types. If tv-a is expressed as a transgene from a promoter active only in specified cell types, the gene transfer achieved by RCAS vector infection will be limited to the cells that use the tissue-specific promoter (8).

Metastasis

Metastasis is a collection of many steps, a process mediated by numerous genes involved in cell adhesion, motility, degradation of the extracellular matrix, and angiogenesis. Many of the genetic manipulations that have improved our understanding of some of these steps were done in the Rip1Tag2 transgenic model of pancreatic cancer (9, 10). There are a few metastatic models in GEMs; however, the theoretical advantage of GEMs in studying metastasis over orthotopic injection models is that one can model all steps of metastasis in the former and only the final steps of metastasis in the latter. In addition, GEMs allow one to study the interplay of immune response with metastasis. More GEMs of metastasis are needed to further elucidate this complex process as metastasis is the cause of death in the majority of cancer patients.

Preclinical Trials in GEMs

Advantages of the many genetically engineered mouse cancer models are that the initiating genetic lesion is known, the mice are immunocompetent, and the tumors develop spontaneously in situ in the appropriate tissue compartment. Complex processes, such as tumor angiogenesis, can be modeled in these in vivo systems. Furthermore, the ability to investigate combinations of agents in native tumors that have not relapsed after previous treatment addresses the limitations of human phase 1 and 2 trials. There are only a few drugs thus far for which GEM preclinical models accord with clinical success. Rego et al. (11) reported that retinoic acid and arsenic work well as anticancer agents in a genetically accurate acute promyelocytic leukemia murine model in accordance with clinical experience. This preclinical model has the same genetic alteration as the respective human tumor. Imatinib, a small molecule that inhibits the Abl, Kit, and platelet-derived growth factor receptor α (PDGFRα) kinases, has proved highly successful in treating chronic myelogenous leukemia (CML), gastrointestinal stromal tumor, and idiopathic hypereosinophilic syndrome (12, 13). Therapeutic responses correlate with inhibition of the relevant mutant kinases (BCR-ABL in CML, Kit in gastrointestinal stromal tumor, and PDGFRα FIP1L1 in hypereosinophilic syndrome). Imatinib also inhibits the growth of hematopoietic malignancies initiated by BCR-ABL in mice (14, 15). These examples support the hypothesis that a tumor model that carries the genetic signature of the native malignancy can recapitulate clinical behavior.

There are several drawbacks to GEMs, including the relatively long length of time needed for GEMs to develop neoplasia and the unpredictable nature of tumor development with regards to frequency and latency of tumor formation. Some GEMs usually have low penetrance. Tumorigenic variance in many transgenic and knockout models is often exacerbated by alterations in genetic background, with some backgrounds being suppressive and others permissive for neoplasia development. This can become a problem for drug efficacy studies unless the genetic background of the GEM is uniform (16). Even with a uniform genetic background, there is still tumor heterogeneity with regards to growth rate, latency, and location. This is due to the natural allelic differences between individuals. The action of these alleles, called cancer modifiers, can sometime cause profound alterations on any aspect of cancer from initiation to therapy response. This heterogeneity is reminiscent of human tumor heterogeneity. To overcome this heterogeneity and reduce mouse numbers and study costs, imaging techniques have been developed to monitor response to therapy. These technologies allow each mouse to be its own control, analogous to human trials. In addition, as these cancer modifiers are difficult to detect in human populations, the mouse has become the predominant model to identify cancer modifiers.

Imaging

Unlike s.c. xenographs that can be monitored easily with calipers, solid tumors in genetically engineered mice usually need to be monitored via imaging. There are several imaging modalities currently available for mice. For anatomic imaging, magnetic resonance imaging (MRI) or micro-computed tomography (CT) is the imaging most frequently used. Whereas micro-CT is the modality of choice for imaging bone, MRI is the tool of choice for other organs. Many murine viral-induced tumors have imaging characteristics similar to human tumors (17). The main disadvantage of MRI is its high cost and time consumption. An
example of a GEM of a glioblastoma multiforme generated via the RCAS/tv-a system, together with its MRI image, is illustrated in Fig. 1.

The uncertainty of initiation and progression of disease, as well as the increasing complexities of GEMs, has necessitated advancement and refinement of noninvasive imaging technologies. These innovative technologies can accurately reveal cellular and molecular changes in the context of the living animal. One of these methods, in vivo bioluminescence imaging, uses internal biological sources of light, luciferases, as reporters of biological change in these models. Optical imaging is based on the transmission of light through mammalian tissues. The sensitivity of detecting photonic signals from within the body is governed by the absorbing and scattering properties of tissues. It can be used to reveal patterns of transcription, levels of protein-protein interactions, extent of tumor burden, sites of metastatic disease, location of immune cell migration, and mechanism of oncogenesis. Bioluminescence imaging has high signal-to-noise ratio and has been validated using biochemical assays. Luciferase and its substrate, luciferin, are nontoxic to mammalian cells and negligible functional differences have been reported between cells expressing luciferase compared with parental cell lines. If a cell type–specific promoter drives luciferase, bioluminescence imaging can be used to count cells (18). In addition, E2F-luciferase transgenic mice have been used as a reporter line to show that the proliferation of PDGF-induced gliomas is dependent on both PDGF receptor activation and mammalian target of rapamycin signaling (19).

**Conclusion**

All modeling systems, extending from xenografts to the most sophisticated GEMs, are imperfect models for human cancer. Evaluating new agents in human patients has many consequential theoretical limitations, including difficulties in studying drug combinations in phase 1 and 2 setting, as well as the fact that patients entered into these trials have refractory or relapsed disease. Although the use of xenograft models to screen potential agents for efficacy remains a mainstay of drug development programs, these systems are biased toward cytotoxic agents and do not correlate well with clinical efficacy. Many GEM strains have been created to develop cancers that accurately recapitulate the genetic, biochemical, and phenotypic features of specific human malignancies. Although it is true that most GEM models...
have not yet been validated against drugs that show some efficacy in the corresponding human cancer, GEMs hold great promise for testing molecularly targeted cancer therapeutics in the near future. There are some specific therapeutic questions that can more easily be tested in GEMs, especially those that affect the interaction between the tumor cells and the tumor microenvironment. Furthermore, the simplistic view of a response to therapy, consisting of tumor shrinkage or prolonged survival in a cohort of mice, is likely to be replaced with more specific questions, such as molecular efficacy, morphologic conversion, and mode of cell death as response to therapy. Lastly, given that GEMs are more accurate models of cancer, they may well be better suited to make correlations between therapy and biology. Overall, harnessing GEMs represents the most promising strategy for translating basic knowledge about cancer pathogenesis into improved treatments (20).

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References

Becher and Holland take the position that GEMs are histologically and genetically accurate models of human cancer, and therefore that these models should be used in cancer drug development. They cite the value of such models with respect to studies of retinoic acid and arsenic in models of acute promyelocytic leukemia and of imatinib in CML, respectively. They seem to miss the point, however, that retinoic acid and arsenic preparations were already known to be of clinical value in acute promyelocytic leukemia long before the construction of the relevant GEM, and therefore how the GEM in any way contributed to the development of these therapies is not evident. With respect to imatinib, their ref. 14 employs only cell lines, and their ref. 15 studies imatinib in a classic xenograft using nonengineered cell lines. We therefore infer that they actually agree with our position that xenograft models, as opposed to GEMs, are quite valuable in providing evidence to support the development of a drug. It is perhaps possible to imagine disease subtypes wherein GEMs more accurately represent in a definite way the target process. Thus, Becher and Holland’s description of a glioblastoma multi-
between mice and man, these GEMs are not suitable for studies of telomerase inhibition. Finally, it has to be noted that some mouse strains used for creating GEMs, such as CD-1 (with 22% lung cancer incidence), have an intrinsically high susceptibility to develop sporadic tumors, which might obscure or overstate targeted gene effects.

Overall, human tumor xenografts established directly from patient explants or cell lines can faithfully represent the genetic complexity of human tumors. If used intelligently as outlined in our review and exemplified in the case of imatinib, (Becher and Holland, ref. 15) where the effect of imatinib on its target was shown as pharmodynamically achievable with the regimen chosen, mouse xenografts will remain the gold standard in cancer drug development.

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