The use of GEM models for experimental cancer therapeutics

Aarthi Gopinathan1 and David A. Tuveson1,*

Introduction
The proposal of new therapeutic strategies for cancer patients has been greatly facilitated by our deepening knowledge of the molecular and cellular etiology of neoplasia. However, the absence of effective in vivo systems that accurately predict clinical efficacy has hindered drug development in oncology. Here, we will briefly discuss the potential advantage of genetically engineered tumor-prone mice compared with xenograft models for the identification of anti-neoplastic agents.

The use of xenografts for preclinical testing
To date, the vast majority of preclinical efficacy studies of various therapeutic agents have been carried out in xenograft models. Xenograft tumor models are generated in immunodeficient mice following the implantation of tumor cells or tumor tissue into ectopic (e.g. subcutis, renal capsule) or orthotopic sites. The commonly stated advantages of tumor xenografts are the ease of model generation and the fact that therapeutic assessment occurs in human cancer tissue as opposed to another species. Furthermore, patient-specific xenografts have recently been described as a means to develop personalized therapies for some malignancies (Rubio-Viqueira et al., 2006; Shu et al., 2008).

Unfortunately, the results obtained from a number of xenograft studies (Boehm et al., 1997; Sarraf et al., 1998) have not translated well into the clinic (Twombly, 2002; Kulke et al., 2002). It is our opinion, therefore, that animal models with better predictive capability will facilitate anti-cancer drug development.

Potential advantages of genetically engineered mouse models
Genetically engineered mouse (GEM) models are a promising alternative to xenograft models for biological and therapeutic investigations. GEM models are generated through the introduction of genetic mutations associated with particular human malignancies. Such mutant genes may be gain-of-function oncogenes or loss-of-function tumor suppressor alleles that are either constitutively or conditionally expressed in mouse models. To date, GEM models have been developed for many common tumor types including lung, prostate, breast, colon and pancreatic cancers, and have been reviewed previously (Frese and Tuveson, 2007). For example, we developed a GEM model of pancreatic ductal adenocarcinoma (PDA) through the targeted expression of an endogenous \( Kras^{G12D} \) allele in murine pancreatic progenitor cells. Such mice developed both preneoplastic and invasive PDA, and cooperated with a concomitant Trp53 mutation to closely recapitulate the human disease at the pathophysiological and molecular level (Hingorani et al., 2003; Hingorani et al., 2005). Several features of tumorigenesis in GEM models are distinct from those found in xenograft models, and these differences may determine the usefulness of either model in preclinical therapeutic investigation.
The neoplastic niche

The initiating genetic lesion in a GEM, through the use of the above-mentioned conditional systems, occurs in the tissue that is relevant to the type of tumor being modeled. As a result, tumor initiation and progression occur in the correct cell type and in the relevant in situ environment. The result may be a pattern of expression whereby mutant cells are surrounded by normal cells, as is the case with tumor initiation in humans. In addition to the initiating molecular events, location of the preneoplasm within a particular cellular microenvironment or region of the organ may affect tumor development. Primary PDAs, for instance, occur very frequently at the head of the pancreas, although the reasons for this are not understood. Tumor initiation in xenografts is very different, consisting of fully transformed, rapidly proliferating tumor cells grown in an environment that is not its normal milieu, and resulting in tumors that differ from corresponding human cancers.

Kinetics of disease development

The kinetics of tumor development in vivo are generally considered to be rather slow, occurring over several years and requiring multiple rounds of selection for cells with additional mutations that render a survival or proliferative advantage. The result is a primary tumor that is heterogeneous and polyclonal. From the therapeutic standpoint, this variation is important because of inherent differences in the populations of tumor cells that may confer a more resistant phenotype to intervention than others. This variability is often lacking in xenografts, as the tumors develop very rapidly and are usually derived from homogeneous or oligoclonal populations of cells. GEM models that have a long latency to tumor development may possess such variability, and will permit the study of pathways and mechanisms of resistance to various therapies. Indeed, our Kras;Trp53 GEM model of ductal pancreatic cancer harbors an underlying chromosomal instability phenotype that may produce a similar genomic heterogeneity to that observed in primary human PDA. Conversely, certain GEM models in which cancers develop rapidly may circumvent some of these stages and may not be effective models of the human disease.

Involvement of the immune system and stroma

One of the major drawbacks of xenograft models is the impairment of the immune system. Numerous studies have reported that the host immune system plays an important role in tumor development (reviewed by Dunn et al., 2004; de Visser et al., 2006). GEM models have the distinct advantage of producing tumors with a well-developed stromal compartment. The stroma consists of extracellular matrix proteins, such as collagen, which lends rigidity to tumors, and a number of different cell types that are recruited during tumor development including activated fibroblasts and immune cells. This allows tumor-microenvironment interactions to be modeled, in particular the role of non-cell autonomous processes and their relevance to tumor development and survival. In preclinical testing, understanding the effect of drugs on the tumor microenvironment, as well as the role of the immune system in the response to therapies, is of primary importance. GEM models can also be used to study the effect of immune-directed therapies.

Tumor structure and vasculature

Accurate GEM models histologically mimic their cognate human malignancy. Tumors from xenograft models, as well as those from a number of early GEM models, are often histologically distinct when compared with tumors found in human malignancies. These
differences in tumor structure and composition may skew the results of drug tests, and affect the response of the tumor to the therapy. For example, PDA xenograft tumors are often exquisitely sensitive to anti-angiogenic agents owing to the neovasculature that develops in xenografts (Bocci et al., 2004; Jia et al., 2005). However, there is less evidence that such a vascular composition is relevant in the cognate human tumor, as exemplified by the clinical failure of anti-vascular approaches in PDA (Kindler et al., 2007).

Metastases
Tumor metastasis represents a major clinical problem for which there are few effective therapies. This process is often difficult to model in xenografts owing to the rapid growth of primary ectopic tumors, although orthotopic implantation followed by resection (Vantyghem et al., 2005) and direct intravascular tumor cell injection can generate models of distant spread. Nonetheless, xenograft models cannot recapitulate the myriad of processes required for metastasis from a primary tumor. GEM models that develop tumors that metastasize from the native site should, therefore, offer the optimal manner to evaluate therapeutics directed against this process.

Tumor monitoring
The development of approaches that accurately describe tumor burden and tumor biology during drug treatment is imperative for successful clinical translation. Given the multiple biological differences between xenografts and autochthonous human tumors, it is therefore not surprising that such methods are routinely developed in clinical investigation rather than during preclinical investigation. Indeed, GEM models should prove advantageous over xenograft models in this regard. For example, high resolution imaging modalities can be developed in GEM models to monitor therapeutic response. These include anatomic methods such as magnetic resonance imaging, computed tomography, and sonography, and functional methods that investigate tissue perfusion or metabolism. Indeed, we have found that, in contrast to PDA xenograft models, our GEM model of pancreatic cancer closely recapitulates the radiographic features of human PDA (K. Olive and D.A.T., unpublished). Additionally, plasma proteomic profiling in GEM models can provide new candidates for tumor detection and monitoring in patients, as recently demonstrated with a mouse model of pancreatic cancer (Faca et al., 2008). As tumor tissue is readily available, GEM models enable the direct correlation between radiological responsiveness, drug levels, and molecular and cellular parameters. This is one of the least explored facets of therapeutic development, owing to the paucity of tumor tissue available from patients undergoing treatment.

Evidence of the usefulness of GEM models in preclinical evaluation
To date, GEM models of breast and lung cancer have been used in preclinical evaluations of therapeutic agents. Similar to clinical experience, lung adenocarcinomas arising as a result of mutant epidermal growth factor receptor (EGFR)-expression regressed on treatment with erlotinib and cetuximab (Ji et al., 2006; Politi et al., 2006). In another study, the response of mammary tumors in p53- and Brca1-deficient mice to the chemotherapeutic agents doxorubicin, docetaxel and cisplatin was evaluated (Rottenberg et al., 2007). Breast tumors in this model demonstrated sensitivity to the chemotherapeutic agents and acquired resistance in a manner that mimicked clinical experience (reviewed in Rottenberg and Jonkers, 2007). Therefore, these early results obtained with GEM models suggest they can provide similar therapeutic responses to those observed in clinical practice.
Although numerous therapeutic studies have been performed in xenograft models, and a few in GEM models, a direct comparison of the two has yet to be reported for any given tumor type. This crucial experiment will improve our understanding of the differences between autochthonous and ectopic tumors, and determine their respective utilities. Such studies will also highlight areas of potential therapeutic interest that have remained unclear so far, providing new and interesting directions for the development of novel therapeutics.

Conclusions

GEM models that accurately model human cancer at both the molecular and phenotypic levels are new tools that are available for experimental therapeutic studies. The crucial initial studies that need to be performed will determine whether GEM models are more successful than xenograft models in predicting the efficacy of approved anti-neoplastic agents. If confirmed, these GEM models should then be used to accelerate the evaluation of novel agents prior to clinical testing.

REFERENCES


