Mouse xenograft models vs GEM models for human cancer therapeutics

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Numerous murine models have been developed to study human cancer. These models are used to investigate the factors involved in malignant transformation, invasion and metastasis, as well as to examine response to therapy. One of the most widely used models is the human tumor xenograft. In this model, human tumor cells are transplanted, either under the skin or into the organ type in which the tumor originated, into immunocompromised mice that do not reject human cells. For example, the xenograft will be readily accepted by athymic nude mice, severely compromised immunodeficient (SCID) mice, or other immunocompromised mice (Morton and Houghton, 2007). Depending upon the number of cells injected, or the size of the tumor transplanted, the tumor will develop over 1-8 weeks (or in some instances 1-4 months, or longer), and the response to appropriate therapeutic regimes can be studied in vivo.

Another type of animal model for studying human cancer is the genetically engineered mouse (GEM) model. The genetic profile of these mice is altered such that one or several genes thought to be involved in transformation or malignancy are mutated, deleted or overexpressed; subsequently, the effect of altering these genes is studied over time and therapeutic responses to these tumors may be followed in vivo. Both athymic nude mice and mouse xenograft models that use human tumor cell lines have been used for decades to increase our understanding of factors affecting tumor growth; however, recent information regarding the key influence of the tumor microenvironment on tumor progression and growth has led to greater reliance on GEM tumor models using immunocompetent mice, as well as use of primary human tumor xenografts in humanized mouse models. In fact, the xenograft models are often regarded as inferior to the GEM models. In this article, I hope to show that each model has its use in cancer diagnostics and in preclinical therapeutic modalities.

Several criteria have recently been suggested for GEM models of human cancers: (1) mice must carry the same mutation that occurs in human tumors; (2) mutations should be engineered within the endogenous locus, and not expressed as a transgene; (3) mutated genes should be silent during embryogenesis and early postnatal development, except for in models of inherited pediatric tumors; (4) mutations should be within the specific target tissues in selected cell types; and (5) mutations must occur in a limited number of cells. Additional ‘desired features’ are that the tumor type and anatomopathology should be as similar as possible to that observed in human tumors, and that tumor development should proceed through the same, or similar, ‘preneoplastic’ stages (M. Barbadic, Keystone Symposium on Inflammation, Microenvironment and Cancer, 2008, and personal communication). Another important criterion, which is difficult to achieve in GEM models, is that the host/tumor environment should be reproducible in the model. Moreover, although mouse tumor models using GEM are highly useful for evaluating the effects of specific mutation, deletion or gene amplification of one or two genes during murine tumor progression, they usually cannot fully reproduce the genetic complexity of human tumors. For example, in humans, malignant melanomas and other tumor types with similar degrees of genetic heterogeneity exhibit an extensive degree of aneuploidy, and the specific gain or loss of genes varies enormously from one cell to another within the same tumor. Thus,
While there are significant strengths to this model, there are innate weaknesses that may profoundly affect the use of these mice for predicting a patient’s response to a therapy.

If one wants to know whether a patient’s tumor will respond to a specific therapeutic regime, one must examine the response of that human tumor, not a mouse tumor, to the therapy. This is where the human tumor xenograft on athymic nude mice, SCID mice, or non-obese diabetic (NOD)/SCID humanized mice can be helpful (Fig. 1). Although some components of the immune system are missing when one chooses nude or SCID mouse models, in athymic nude mice, the B cells, dendritic cells and granulocytes are all relatively intact, and there is a compensatory increase in both natural killer (NK)-cell activity and tumoricidal macrophages in these mice. Moreover, one can argue that, by the time these metastatic lesions are surgically removed or biopsied, the tumor has already escaped immune surveillance and cell killing by the immune cells.

In this editorial I will discuss the advantages and disadvantages of human tumor xenografts, compared with GEM models, as a method of analyzing the potential responses of patients’ tumors to therapy (Fig. 1).

There are several key advantages of using human tumor xenografts to examine therapeutic responses to drugs: (1) one can use the actual human tumor tissue, featuring the complexity of genetic and epigenetic abnormalities that exist in the human tumor population; (2) human tumor xenografts can be used to aid in the development of individualized molecular therapeutic approaches; (3) results can be obtained in a matter of a few weeks from a human tumor biopsy regarding response to therapy, whereas the GEM models often require as long as a year to develop prior to drug therapy; (4) multiple therapies can be tested from a single tumor biopsy; (5) data from tissue microarrays and genetic microarrays can be readily obtained from the human biopsy and xenograft tissue, before and after drug therapy, for extensive analysis before the patient is subjected to therapy that may not work; (6) orthotopic xenografts can be appropriately placed to reproduce the organ environment in which the tumor grows, so that the effect of the tumor on its microenvironment can be modulated, albeit with the exception of certain T-cell populations; (7) stroma from the human tumor microenvironment can be included in the xenograft to more completely mimic the human tumor microenvironment; and (8) xenografts using NOD/SCID mice that have been ‘humanized’ by injection of peripheral blood or bone marrow cells, allow for an almost complete reconstitution of the immune response to the tumor. Xenografts using human cell lines to test drug responses do not often correlate with clinical activity in patients (Kerbel, 2003). By contrast, when primary tumors are used as an orthotopic xenograft, there is a stronger predictive response value, especially when a clinically relevant drug dosage is used (Johnson et al., 2001; Kerbel, 2003; Scholz et al., 1990). There are three different types of response to therapy that can be evaluated: effect on the growth rate of the tumor, effect on tumor shrinkage/regression, and survival. The effect of a drug on the rate of tumor growth or cytostasis has been reported to often be more predictive of a clinical response than tumor shrinkage/regression (Kelland, 2004). Moreover, subcutaneous tumor models that are not orthotopic and do not represent appropriate sites for human tumors are not predictive when used to test responses to anti-cancer drugs (Killion et al., 1998). A challenge presented with orthotopic models, as compared with subcutaneous models, is the difficulty of following tumor growth. However, the recent development of new magnetic resonance imaging (MRI) and micro-imaging techniques may minimize this problem.

There are several other disadvantages and challenges of using the mouse xenograft model to monitor and/or predict therapeutic responses in cancer. Orthotopic tumor
models are time consuming, expensive and technically challenging. In addition, if athymic nude or SCID mice are used, the lymphocyte-mediated response to the tumor is lost, i.e. nude mice lose certain T-cell responses and SCID mice lose both their T- and B-cell responses. However, these immunological deficits can, in principle, be largely overcome by grafting human tumors onto ‘humanized’ NOD/SCID mice. This greatly reduces many of the drawbacks of the orthotopic human tumor xenograft models for studying therapeutic response. However, full restoration of the immune system in the ‘humanized mouse’ is not possible, since restoring HLA class I- and class II-selecting elements in T-cell populations remains a challenge (Bernard et al., 2008). Moreover, to perform these experiments the newborn mice must be irradiated and then engrafted with human CD34+ hematopoietic stem cells from human umbilical cord blood. The timing of obtaining cord blood, irradiating newborn mice and verifying the humanized phenotype of the NOD/SCID mice after engraftment, makes this procedure quite cumbersome, but highly valuable.

Fig. 1. Types of murine model for studying human cancers.
In spite of the disadvantages of the xenograft model for predicting clinical response to therapy, there are a number of important successes. For example, xenografts of multiple myeloma cell lines into syngeneic mice respond to the proteasome inhibitor, bortezomib/VELCADE®, which has shown significant promise for the treatment of multiple myeloma (LeBlanc et al., 2002; Moreau et al., 2008; Oyajobi and Mundy, 2003). The combination of bortezomib and melphalen was first demonstrated as effective for treatment of multiple myeloma in preclinical xenograft trials, and this led to success in clinical trials followed by a recommended new standard of clinical care for multiple myeloma patients over 65 years of age (Mateos et al., 2006; Mitsiades et al., 2003). Herceptin was shown to enhance the anti-tumor activity of paclitaxel and doxorubicin against HER2/neu-overexpressing human breast cancer xenografts, and this led to subsequent successful clinical trials (Baselga et al., 1998; Sporn and Bilgrami, 1999). Neutralizing antibodies targeting vascular endothelial growth factor receptor 2 (VEGFR2) in combination with paclitaxel were shown to be effective in inhibiting tumor growth and inhibiting metastatic spread in an orthotopic xenograft model (Davis et al., 2004). This groundwork was followed by development of bevacizumab, a humanized monoclonal antibody that targets vascular endothelial growth factor A (VEGF-A). Bevacizumab was effective in Phase III clinical trials for colorectal and renal carcinoma and received FDA approval in 2004 (Hurwitz et al., 2004; Yang et al., 2003). Moreover, mouse xenograft models are useful for anticipating toxicity from targeted therapies, and, in other cases, to identify possible predictive biomarkers of target modulation. Although these are only a few examples of the successful use of xenograft studies, clearly, for many types of human tumors, the information learned from mouse orthotopic xenograft studies using human tumors has led to information that has been translated into successful clinical trials.

The advantages of the GEM are that: (1) the mice are immunocompetent, such that the tumor microenvironment can be mirrored as much as possible in a murine tumor model; (2) specific genetic abnormalities that are present in human tumors can be reproduced, in an inducible manner, at specific ages in the tissue-type of origin; (3) the stages of tumor progression can be studied over time; and (4) several therapeutic approaches can be explored at various stages of tumor development. Genetic models are also useful in humanized mice, where human genes, such as the cytochrome P450 genes or human tumor antigens, are expressed in mice to follow drug metabolism or immunological responses to the tumor (Talmadge et al., 2007). The disadvantages of the GEM are, first, that the complexity of the human tumor cannot be reliably mimicked and, second, mouse tumors are not human tumors and do not often predict what will happen in the human tumor with regard to therapeutic response. We can cure many mouse tumors, but there is not a direct correlation between response in the mouse and response in the clinic.

In summary, both the orthotopic human tumor xenograft and the GEM models are useful for enhancing our understanding of cancer development and treatment. Each has its strengths and limitations, with the orthotopic human tumor xenograft being excellent for predicting drug response in human tumors, and the GEM model being best for examining the role of specific genes in tumor development and progression. Whichever model is used to predict clinical response in patients, it is important to get at least a 50% inhibition in tumor growth to achieve a qualified ‘response’ to therapy, and to use clinically relevant dosages of therapeutic agents and monitor survival. In addition, it is important to determine whether tumor growth returns when the drug is discontinued, and, if so, whether the re-growth is faster when treatment is paused compared with before the treatment began. If this is the case, in spite of any response
to drug therapy, the rebound effect advises against use of that drug treatment regime for tumor types exhibiting a rebound effect. In conclusion, we do not have ideal murine models of human tumors, but must learn to interpret our data within the framework of the limitations of the assay used.

REFERENCES


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