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Human Primary Tumorgraft Models: Comparisons with Traditional Oncology Pre-Clinical Models and The Clinical Relevance and Utility of Primary Tumorgrafts in Basic and Translational Oncology Research

David H. Lum[1], **Cindy Matsen**[2], **Alana L. Welm**[1], and **Bryan E. Welm**[1,2,3]

[1]Department of Oncological Sciences Huntsman Cancer Institute University of Utah Salt Lake City, UT 84112

[2]Department of Surgery Huntsman Cancer Institute University of Utah Salt Lake City, UT 84112

Abstract

Laboratory models that accurately replicate human tumors are integral to cancer research. However, comparative studies between commonly employed laboratory models and human tumors have demonstrated that some models have molecular and genomic alterations dissimilar to the cancer type they attempt to replicate. In contrast, several recent comparative studies suggest that patient-derived tumors grown in mice maintain many of the important characteristics of the original tumor and represent an important tool for the development of new cancer therapeutics. Many models are now available for mechanistic and therapeutic research, and each system has both strengths and weakness. This review will discuss the advantages and disadvantages of the most commonly used models of cancer and highlight advances in the generation and use of patient-derived tumorgrafts.

Keywords

Cancer Models; Tumorgraft; Xenograft; Orthotopic Transplantation; Genetically Engineered; Mouse Models; Patient-derived Tissue

An ongoing challenge in oncology research is the development and application of experimental models of human tumorigenesis, disease progression, and therapeutic response. Such model systems are critical to achieve a better understanding of cancer biology and to improve cancer treatments. The ideal model(s) would recapitulate human tumors both phenotypically and on a molecular level: they would grow in the correct developmental and anatomic context, surrounded by the appropriate tumor microenvironment and an intact immune system; they would faithfully reproduce the assortment of genetic alterations found in human tumors; they would replicate the full spectrum of tumor pathologies and subtypes; and they would exhibit patterns of metastasis observed in patients. However, no model is perfect and even state-of-the-art models fail in one or more of these areas. Thus, it is often necessary to utilize several models in an effort to obtain a comprehensive understanding of cancer mechanisms. Here, we will review the most frequently used models of cancer, including genetically engineered mouse models and xenografts derived from either cancer cell lines or primary tumor tissue (known as

[3]Corresponding author: Department of Surgery Huntsman Cancer Institute University of Utah 2000 Circle of Hope Salt Lake City UT 84112 (801)-587-4622 bryan.welm@hci.utah.edu.

tumorgrafts). We will focus on the advantages and disadvantages of each model in terms of its ability to replicate the human disease and its utility in preclinical studies.

Genetically engineered mouse models of cancer

Since the advent of gene-targeting and transgenic methodologies, there has been continuous growth in the number of genetically engineered mouse models (GEMMs) of cancer, leading to the generation of models that closely recapitulate important aspects of human tumors (Resor et al., 2001; Cheon and Orsulic, 2011). GEMMs hold several advantages for studying molecular mechanisms that drive cancer behavior. First, mice can be generated that harbor specific genetic mutations that are known to be associated with human tumors, and techniques taking advantage of DNA recombination and inducible gene expression allow flexibility to induce the cancer initiating mutation(s) in both a tissue-dependent and time-dependent fashion (Van Dyke and Jacks, 2002). Second, tumor development in GEMMs occurs in immune-competent hosts and thus maintains the complex interactions that occur between tumor cells and the immune system (Egeblad et al., 2010). Third, tumor development is spontaneous and often proceeds through stages of progression that can be similar to that observed with human tumors (Lin et al., 2003; Yamada and Mori, 2007). Finally, as the number of GEMMs continues to grow, it has become routine to examine the influence of combinations of mutations on tumor initiation and progression. Thus, continued refinement of molecular and genetic techniques has made GEMMs particularly suited to dissecting the role of specific molecular events in tumorigenesis and/or disease progression.

While GEMMs clearly play a crucial role in understanding basic mechanisms of cancer biology, and will be increasingly utilized in translational research and drug screening (Sharpless and Depinho, 2006; Cheon and Orsulic, 2011), these models do have some disadvantages as models of human cancer. First, despite numerous similarities, mouse tumors are fundamentally different from human tumors both in terms of genetic makeup and physiology. Second, the dominant approach of using reverse genetics to develop GEMMs often restricts investigation to genes already known or suspected to play a role in cancer. Third, the experimental manipulation of one or two driving oncogene(s) or tumor-suppressor gene(s), which are often over-expressed to non-physiological levels or deleted throughout an entire tissue type or organism, may facilitate tumor development in ways that do not accurately represent the natural evolution of human tumors over the span of decades. Thus, while we can generate tumors quickly in mice by exploiting expression or deletion of many of the same genes that are altered in human tumors, the tumors may be different than those occurring in patients (Rangarajan and Weinberg, 2003; Herschkowitz et al., 2007).

Cancer cell lines as derivatives of human tumors and models for drug discovery

Cell lines have become a nearly ubiquitous feature of cancer biology laboratories in both academia and the pharmaceutical industry, and the foremost cancer model used for high-throughput screens for drug discovery. Their cost-effectiveness, indefinite lifespan, ease of use, limited cellular heterogeneity, and genetic manipulability have contributed to their dominance as experimental tools in the laboratory setting. Because they are derived from human cancers, cell lines exhibit many intrinsic features of cancer, sharing many gene expression patterns and genomic alterations with primary human tumors (Forozan et al., 2000; Ross and Perou, 2001; Neve et al., 2006; McConkey et al., 2010). Moreover, cell lines can display dependence on well-known and clinically relevant cancer growth pathways, including estrogen-mediated signaling in MCF7 breast cancer cells, HER2 in SKBR3 breast cancer cells, and BRAF in A375 melanoma cells (Lippman et al., 1975; Coezy et al., 1982; Lupu et al., 1990; Karasarides et al., 2004).

Several additional characteristics of cell lines such as their adaptation to cell culture, limited heterogeneity and high proliferation rates have made them extensively used in drug discovery programs. One example of the utility of cell lines in the drug discovery process is illustrated by the National Cancer Institute's (NCI) anticancer drug screening program (Shoemaker, 2006). Established in the 1980s, the intent of this program was to screen a panel of 60 cell lines, derived from cancers of nine tissues, in order to identify and prioritize compounds with selective anticancer activity. This approach has had a significant impact on cancer therapeutics with the screening of over 100,000 compounds to date, and has played a role in the development of important anti-cancer drugs such as taxol, trastuzumab, bevacizumab and bortezomib (Cragg, 1998; Richmond and Su, 2008).

Cancer cell line xenografts as *in vivo* models for cancer

Cell lines are highly simplified models of human cancer that by themselves are not able to replicate the complexity of the tumor microenvironment. In culture, cell lines lack stroma components including; blood and lymphatic vessels, associated immune cells, fibroblasts, and the presence of a complex extracellular matrix (Egeblad et al., 2010). Since all of these components are known contributors to tumor progression and metastasis, more complex *in vivo* systems are required to accurately model cancer. Tumor xenografts, which are usually generated by transplantation of human cancer cell lines into a host organism such as immune-compromised mice, provide a method to model human tumors *in vivo*. Cell line xenografts are particularly useful to investigate the consequences of genetic manipulation of human tumors or the effect of drug treatments on tumor growth. In addition, cell lines transfected with fluorescent or bioluminescent expression constructs are widely used to monitor tumor progression and metastasis using non-invasive imaging techniques in living animals. Thus, cell line xenografts have become one of the most effective methods for studying human cancers *in vivo*, and virtually every clinically approved anti-cancer drug has been evaluated in human tumor xenograft models (Kerbel, 2003).

Cell line xenografts as predictors of drug response in patients

The success of many drugs in xenograft models followed by their failure in clinical trials for patients calls for increased scrutiny of the reliability of these models to predict the clinical success of therapeutics (Holmgren et al., 1995; O'Reilly et al., 1996; Boehm et al., 1997; Sarraf et al., 1998). A retrospective analysis by NCI comparing preclinical xenograft data with Phase II clinical trial data for 39 compounds revealed there was no correlation between xenograft and clinical effectiveness when tumor types of the model and patient were matched. However, if a compound induced a response in at least one third of the xenograft models, irrespective of tumor type, then the models were predictive of clinical response (Johnson et al., 2001). A large part of the discrepancy in drug response between xenografts and clinical trials may result from differences in pharmacokinetics, clinically equivalent doses (CED) and varied treatment routes and schedules (Kerbel, 2003). In mouse xenograft models, a compound's efficacy is often assessed at a maximum tolerable dose (MTD) rather than a CED. A compound's MTD is normally determined by escalating dosage in genetically identical and healthy mice until dose-limiting parameters such as weight loss, lethargy, or other morbidities are observed. In contrast, during clinical trials genetically diverse individuals of different ages, health, and tumor burden are assessed for numerous adverse drug effects. Moderate adverse effects in even a small percentage of patients can be considered dose limiting and result in lower dosage recommendations for all patients. Finally, routes and schedules that are used for treating mice, such as daily intravenous treatments, can be inconvenient for patients and costly in a clinical setting. These differences can lead to an overestimate of a compound's therapeutic potential during preclinical studies.

Optimizing dosages and schedules used in xenografts around a CED should reduce discrepancies between preclinical studies and clinical observations.

Challenges with cell lines as models of human cancer

Although cell lines are highly amenable for modeling human cancer and can be grown both *in vitro* and *in vivo*, they also carry disadvantages. While cell lines usually exhibit molecular features similar to human tumors (Neve et al., 2006; Kao et al., 2009), it has also been observed that their genomes diverge when extensively passaged, which is not surprising considering the inherent genetic instability of most human cancers and unique selective pressure imposed by cell culturing. In comparison to their low passage counterparts, high-passage cells can acquire alterations in gene expression, chromosome arrangements, karyotype, differentiation markers, and growth rates (Hughes et al., 2007). Moreover, studies have shown that several cell lines commonly used in research are either misrepresented as a specific tissue type, or are contaminated with other cell lines. Since it is common practice to obtain cell lines from other laboratories without performing a thorough quality control assessment, it is likely that these issues are more pervasive than currently appreciated (Burdall et al., 2003; Hughes et al., 2007). Thus, it is important that researchers verify cell lines as appropriate models for their experimental question, and validate results in more than one cell line in order to ensure observations are more widely applicable.

While many characteristics make cell lines well suited for use in high-throughput screens, cell lines also exhibit qualities that may negatively impact the capability to identify diverse anti-cancer compounds. First, the high growth rate of cell lines may bias toward the discovery of anti-proliferative drugs, and impede discovery of compounds affecting unique, growth-independent pathways. Evidence for this bias is demonstrated by the fact that cell proliferation is the dominant pathway targeted by current chemotherapies. Second, cancer is a heterogeneous collection of diseases, and the full spectrum of such pathological diversity is not adequately represented by the current collection of cell lines. Many lines were clonally-derived from late stage metastatic cancers, and relatively few lines originated from pre-neoplastic disease. Due in part to their clonal origins and in part to the selective pressures of culturing *in vitro*, cell lines exhibit limited cellular complexity compared with primary tumors, and are devoid of supporting cell types such as cancer associated fibroblasts or immune cells, which are known to affect tumor growth (de Visser et al., 2006; Kalluri and Zeisberg, 2006). Thus, use of cell lines as the dominant model for cancer research and drug screening may hinder investigation of early events in transformation and the discovery of compounds targeting the diverse mechanisms that drive cancer progression.

Tumorgrafts as improved *in vivo* models for cancer

The increasing use of microarray and other genome-wide analyses over the last decade has generated a renewed appreciation of the molecular heterogeneity of cancer and its impact on patient prognosis. This has resulted in another possible explanation for the poor translation of drug responses from cell line xenografts to the clinic, resulting in a demand for models that more closely reflect the clinical diversity of cancer.

Primary tumorgraft models, which are generated by transplanting either intact tumor fragments or cancer cells isolated directly from patients into immune-deficient mice, offer an opportunity to enhance the diversity of human cancer models and limit the undesirable effects associated with cell culture. Over the past several decades, a number of studies have suggested that tumorgrafts maintain the histology, gene expression patterns and genomic variation of patient tumors. However, few studies have performed a comprehensive molecular and genomic comparison between patient tumors and tumorgrafts. The recent availability of relatively low cost platforms for global gene expression analysis and whole

genome sequencing has created opportunities to comprehensively examine molecular similarities between patient tumors and their matched tumorgrafts. For example, a recent study by Ding *et al.* performed whole-genome analysis of a patient's peripheral blood, primary breast tumor, and brain metastasis with a tumorgraft derived from the primary tumor (Ding et al., 2010). These data revealed that most somatic mutations and genomic variations were preserved between the tumor, metastasis and tumorgraft. Moreover, the tumorgraft maintained all of the primary mutations of the original tumor, which suggests that at least early passage grafts have sufficient genomic stability to appropriately retain the molecular features of the originating tumor. While this particular study did not assess the genomic stability over several rounds of serial transplantation, a recent comprehensive analysis by our group showed that serially-transplanted tumorgrafts do exhibit histological and molecular stability when compared to the patients' original tumors (DeRose et al., 2011). In this study, DeRose *et al.* analyzed 12 primary tumorgrafts derived from different breast cancer patients for clinical markers (ER, PR, HER2), metastatic potential, intrinsic subtype by microarray gene expression, hormonal dependence, DNA amplification and deletion, and histology for up to five serial transplantations. The data demonstrated that serially-transplanted tumorgrafts were highly similar to the original patient tumors. For example, unsupervised hierarchical clustering of microarray gene expression data using approximately 1900 genes showed that all but one serially-transplanted tumorgraft clustered together with the original patient tumor. Interestingly, the patient tumor that did not cluster with its tumorgraft generated both lymphomas and adenocarcinomas upon transplantation, suggesting that this tumor was heterogeneous and composed of different cancer types. These comprehensive analyses demonstrate breast tumorgrafts can accurately reflect the molecular and clinical heterogeneity of tumors for at least five rounds of transplantation. However, general adoption of tumorgrafts as preferred model systems will require further studies to evaluate whether genomic and molecular stability is maintained using different cancer tissues and over more rounds of serial transplantation and/or experimental manipulation.

A variety of different methods have been used to establish tumorgrafts for various cancer types, resulting in varying success in their ability to replicate clinical disease. For example, the importance of the site of transplantation is considerable since tumor growth, metastasis and drug pharmacokinetics are affected by tissue physiology. Orthotopic transplantation engrafts the tumor directly into the relevant organ in the mouse, and appears to provide the best physiologic cancer models. However, performing orthotopic transplantation in certain tissues can be challenging and inefficient, particularly when studies involve large cohorts of mice. Often alternative methods that provide relatively easy access to tissue, such as subcutaneous and renal capsule transplantation, are used but these are not natural sites of tumor growth or metastasis, and their relevance as cancer models has been criticized. In addition to differences in response to drugs, subcutaneous engraftments can have divergent metastatic properties, such as loss of metastatic potential or dissemination to sites that are not clinically relevant. This is not surprising since tissues are composed of microenvironments with considerable differences in not only the type of extracellular matrix proteins (ECM) present, but also their density and level of crosslinking (Egeblad et al., 2010). Tumor cells evolve within their particular microenvironment and develop metastatic capability by expressing cell surface receptors and enzymes that enhance their adhesion and invasion specifically within that tissue. These same proteins can also contribute to a tumor's preference for metastatic seeding (Minn et al., 2005a; Minn et al., 2005b; Langley and Fidler, 2007). Thus, tumor cells may not be poised to invade and metastasize when transplanted into non-native microenvironments, and may therefore have to evolve different mechanisms for dissemination.

For over two decades, xenograft models have been used to study human colorectal cancer. Initially, xenografts were derived by the establishment of cells in culture from primary

human colorectal carcinoma tumors followed by their injection either into the subcutis, caecum or spleen of nude mice (Giavazzi et al., 1986a; Giavazzi et al., 1986b; Morikawa et al., 1988a; Morikawa et al., 1988b). These early studies not only demonstrated the feasibility of generating xenograft tumors from primary human colorectal cancer cells, but also established the necessity of the orthotopic microenvironment for proper recapitulation of tumor development. It was observed that tumors injected subcutaneously failed to metastasize while those that were orthotopically transplanted into the caecum metastasized to the lymphatics, liver and lungs (Giavazzi et al., 1986a; Bresalier et al., 1987; Fidler, 1991; Fu et al., 1992; Pocard et al., 1996). Similar results have been found with cells derived from several human tumors including breast, lung, pancreatic and bladder cancers (Fu et al., 1991; Kubota, 1994). Following initial xenograft studies with dissociated tumor cells, Fu *et al.* suggested that transplantation of intact tumor specimens might better reflect the original properties of the cancer by avoiding the disruption of the tumor microenvironment (Fu et al., 1991; Fu et al., 1992; Furukawa et al., 1993b). Upon orthotopic implantation of tumor fragments from 20 patients into colons of nude mice, 65% of the mice developed tumors with varying degrees of local growth, lymph node, liver and abdominal metastasis (Fu et al., 1991). Further studies have suggested a 40-60% take rate using intact tumor samples (Damia and D'Incalci, 2009). It was subsequently demonstrated that orthotopic transplantation of the human colon tumorgraft line, COL-2-JCK, as intact tissue resulted in 100% take rate, with extensive local tumor growth and a high incidence of metastases to the regional lymph nodes, peritoneum, liver, and lung (Furukawa et al., 1993b). However, injection of a cell suspension of this same tumorgraft line yielded no metastases and significantly reduced local tumor growth. In fact, the improvement of orthotopic implantation of tumor fragments over the transplantation of dissociated cells is not unique to colon cancer, but has also been observed with many other forms of cancer (Hoffman, 1998).

Tumorgrafts as models for drug development and testing

Although it is becoming apparent that tumorgraft models may be the best representation of human cancers *in vivo*, a key question is whether tumorgrafts will be more accurate at predicting drug efficacy compared to the simpler, less expensive models employing cancer cell lines. As yet, there have not been enough studies to allow us to definitively answer this question. However, several studies have yielded promising results demonstrating the value of tumorgraft models as preclinical models for cancer treatment strategies.

In one study, small cell lung cancer cells growing in an orthotopic site replicated clinical observations by exhibiting a significant response to cisplatin, but limited response to mitomycin C. In contrast, the same cells growing in subcutaneous tissues exhibited an opposite response to these drugs (Kuo et al., 1993). In the case of colorectal cancer, a disparity in efficacy was seen when clinical data was compared with data from *in vivo* subcutaneous xenografts treated with 5- fluorouracil (5-FU) and mitomycin C (MMC), two of the most commonly used therapies (Carter SK, 1974). In both of these studies, the subcutaneous xenografts overestimate the efficacy of therapy compared to actual response rates in patients (Inaba et al., 1988; Kubota et al., 1988; Furukawa et al., 1991). In a separate study using orthotopic implantation of intact patient colon tumor samples, Furukawa *et al.*, found that although 5-FU and MMC both had antitumor effects on cecal tumor growth, neither affected the formation of liver metastases at the maximum tolerated dose (MTD) (Furukawa et al., 1993a). Thus, the results of this study more closely reflected the clinical efficacy of 5-FU and MMC than experiments using subcutaneous xenografts. Furthermore, the differential efficacy of 5-FU and MMC against the primary cecal tumor and liver metastases emphasizes the importance of metastatic models for modeling the therapeutic response of advanced disease.

Tumorgrafts also hold the possibility for development of assays in which to screen potential new therapies across a broad spectrum of tumor types. This has been most widely employed in pediatric cancers using the Pediatric Preclinical Testing Program (PPTP) supported by the National Cancer Institute (Houghton et al., 2007). Based on retrospective studies, xenograft models of childhood cancers have been quite accurate in identifying clinically active agents and effective drug combinations (Peterson and Houghton, 2004). Thus, the PPTP was initiated to identify new anticancer agents that have the potential for significant activity when clinically evaluated against selected childhood cancers. The PPTP panel is made up of over 80 xenograft lines derived from Wilms tumor, sarcomas, neuroblastoma, brain tumors, rhabdoid tumors (CNS and renal), and acute lymphoblastic leukemia (ALL). The xenograft lines in the PPTP have been extensively characterized and display overall high transcriptional similarity to their primary tumors and maintain gene copy alterations (Whiteford et al., 2007; Neale et al., 2008). To examine the predictive value of the PPTP xenograft panel, Houghton *et al.* treated the panel with two standard chemotherapeutic agents, vincristine and cyclophosphamide (Houghton et al., 2007). Both agents displayed broad-spectrum activity and generally recapitulated their activity against specific childhood cancers. These results are promising and currently the PPTP has the capacity to screen 10-15 compounds per year.

Another advantage of patient-derived tumorgrafts is that they have the unique potential of being generated and studied in the laboratory concurrent with patient treatment in the clinic, offering the opportunity of personalized medicine for the patient. For example, tumorgrafts can be transplanted for *in vivo* therapeutic studies in mice or cultured in either two- or three-dimensional drug screening assays. Evidence supporting the value of tumorgrafts in directing clinical treatment comes from work by Hidalgo *et al.*, where a panel of tumors resected from 14 patients with refractory advanced cancers were propagated in immune-deficient mice and treated with 63 anticancer agents (Hidalgo et al., 2011). Based on the therapeutic response in the tumorgraft models, effective treatment regimens were identified for 12 patients. An objective response rate of 88% for treatments deemed effective by the model and tested in the patients is significantly greater than the 10% expected with Phase I agents (Horstmann et al., 2005; Hidalgo et al., 2011). There are obvious limitations to this approach, however, including the need for a relatively large amount of fresh tumor tissue from the patient, significant cost, the sometimes lengthy tumorgraft propagation time, and the obligatory lack of a functional immune system. Still, the success of this research exemplifies the clinical benefits of tumorgraft models: they closely replicate primary tumors, they expand the spectrum of cancer subtypes that can be modeled, and they offer new possibilities for drug discovery and personalized medicine.

Conclusions

While the perfect preclinical cancer model may be unattainable, there are many available models that can assist in the pursuit of a better understanding of cancer and better clinical care. Primary tumorgrafts hold the promise of enhanced clinical predictive potential and may be the best representation of human disease as well as more accurate predictors of clinical response. The true predictive nature of these models have yet to be borne out in clinical trials, but as drugs tested in these models begin moving into the clinical testing phase, we await the results with great anticipation.

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