Mouse models of cancer as biological filters for complex genomic data

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Genetically and pathologically accurate mouse models of leukemia and lymphoma have been developed in recent years. Adoptive transfer of genetically modified hematopoietic progenitor cells enables rapid and highly controlled gain- and loss-of-function studies for these types of cancer. In this Commentary, we discuss how these highly versatile experimental approaches can be used as biological filters to pinpoint transformation-relevant activities from complex cancer genome data. We anticipate that the functional identification of genetic ‘drivers’ using mouse models of leukemia and lymphoma will facilitate the development of molecular diagnostics and mechanism-based therapies for patients that suffer from these diseases.

Introduction

Treatment and diagnosis of leukemia and lymphoma are rapidly moving towards molecular diagnostic assignments and mechanism-based therapies. Recent improvements in the supportive care that accompanies the conventional chemotherapy of cancer have enhanced treatment outcomes and enabled more-intensive therapies. As we work towards further improvements, it has become clear that the next major step forward will involve the development of mechanism-based therapies, and the assignment of individual patients to the most suitable therapeutic regimen. The latter relies on the identification and use of meaningful molecular markers to complement pathological diagnoses, and is often referred to as ‘personalized medicine.’ Recent examples include the use of kinase inhibitors in the treatment of myeloproliferative diseases as well as solid tumors (for a review, see Sawyers, 2004). Notably, innovative clinical trials currently in progress include patient-specific mutation detection as a guide to therapy (Arteaga, 2006; Solit et al., 2006; Levine and Carroll, 2009). Many of these breakthroughs in the development of new therapies were brought about using new information about the genetic mechanisms that drive these cancers. Mouse models facilitated this development by enabling studies of oncogene–tumor-suppressor interactions and genotype-response relationships in vivo (for a review, see Lowe et al., 2004). Hence, insights into cancer genetics can lead to major advances in treatment development.

Genomic analyses of cancer cells are now unraveling the complexity of the genetic changes that contribute to cancer. New technologies have revealed cancer-specific changes in mRNA and microRNA expression as well as genomic rearrangements, such as chromosomal gains and losses (for a review, see Hawkins et al., 2010). The resulting datasets are often complex, and this probably reflects the complicated pathways involved in converting a normal cell into a cancer cell. However, it is unlikely that all changes detected in the genomes of cancer cells are functionally important, and many might be random. Hence, there is a signal-to-noise problem in separating the ‘drivers’ from the ‘passengers’ at the level of genetic changes. To some extent, an increasing number of analyses of genomes from tumor versus normal cells can help define recurrent lesions and thereby act as a first filter of complex genomic data. However, in many cases even a large number of analyses cannot directly pinpoint the activities that are targeted by genomic lesions or provide functional insight into their role in tumor biology. Mouse models of cancer have an emerging role in enabling the functional annotation of complex cancer genome data obtained from human patients.

In this Commentary, we focus on hematopoietic malignancies for which accurate mouse models have been reported. For example, several genetically engineered mouse models of lymphoma recapitulate the genetics and pathology of Burkitt’s lymphoma (Eμ-Myc) (Adams et al., 1985), follicular lymphoma (vavP-Bcl2) (Egle et al., 2004), diffuse large B-cell lymphoma (DLBCL) (µHA-BCL6) (Cattoretti et al., 2005a; Cattoretti et al., 2005b) and mantle-cell lymphoma (Eμ-CyclinD1) (Bodrug et al., 1994; Gladden et al., 2006). Typically, these models express oncogenes that are involved in human lymphoma, such as Myc, Bcl2 and Bcl6, placed under control of immunoglobulin (Eμ or µ) or broad hematopoietic (vavP) enhancer elements. In this way, they recapitulate the effects of common chromosomal rearrangements found in the corresponding tumor types. Furthermore, mouse models of acute leukemias driven by retrovirally expressed oncogenes have been established. Specifically, these include B-cell and T-cell acute lymphatic leukemia (B-ALL and T-ALL, respectively), acute myeloid leukemia (AML) and chronic myeloid leukemia (CML). These models are driven by retroviral transduction of hematopoietic progenitors driving the expression of the BCR-ABL fusion (Pear et al., 1998), Notch-induced T-ALL (Pear et al., 1996) or AML driven by a MLL-ENL fusion (Lavau et al., 1997). In addition, a rare germline mutation targeting miR-16 in the New Zealand black mouse strain makes for a particularly intriguing model of chronic lymphatic leukemia (Raveche et al., 1979; Raveche et al., 2007). These widely used models have shed light on key genes and pathways involved in leukemia and lymphoma.

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Mouse models of hematopoietic malignancies are especially amenable to genetic studies

Conventional crosses and adoptive transfer of genetically modified hematopoietic progenitor cells (see below) enable the study of genetic lesions in a physiological context. Crossing models of hematopoietic malignancies to transgenic or knockout mice has revealed the function of many tumor suppressor genes and the involvement of cellular ‘fail-safe’ mechanisms, including apoptosis and senescence, in oncogenesis and responses to treatment (Eischen et al., 1999; Schmitt et al., 1999; Schmitt et al., 2000; Cleveland and Sherr, 2004). However, cross-breeding to genetically engineered mice is very costly and limits the applicability of this approach. Adoptive transfer approaches have enabled the rapid generation of genetically defined tumors without the need for additional transgenic or knockout animals (Schmitt et al., 2002; Wendel et al., 2004). Briefly, adoptive transfer experiments involve the isolation of hematopoietic progenitor cells from bone marrow or from fetal liver, and their subsequent modification using retroviruses or other methods during a brief period of in vitro culture. These modified progenitor cells are then used to reconstitute the hematopoietic system of irradiated, syngeneic animals, resulting in chimeric animals that have a hematopoietic system derived from the modified donor cells. This approach is highly versatile and enables both gain- and loss-of-function studies of specific genes using cDNA or short-hairpin RNA (shRNA) expression, respectively. Some cases in which adoptive transfer approaches have been successful include the genetic dissection of oncogenic signaling pathways in vivo and structure-function analyses that defined functional domains within oncogenes (Wendel et al., 2007; Mavrakis et al., 2008). Clearly, there are logistical and conceptual shortfalls with each of these models – for example, the ubiquitous and/or non-physiological expression levels of transgenes, subtle differences in the timing and location of transformation in the models, and general species-specific differences between human and mouse cells. Nevertheless, these models are helpful for isolating specific genetic changes and defining their impact on cancer phenotypes in vivo.

The ease of carrying out genetic studies in mouse models of hematopoietic cancers provides an opportunity to decipher the genomic changes seen in human cancers. Briefly, datasets derived from the genomic analysis of human cancer are typically complex and, although statistical and computational analyses can be used to narrow down candidate genes that are commonly found to be aberrant, they do not provide direct evidence of cancer-relevant biological activities. In this regard, mouse models can help to functionally annotate human genomic data. For example, in a candidate-gene approach, genomic data are first analyzed for the most common changes, and then related to established knowledge about gene functions. The most promising candidate genes are then directly tested for their potential biological role in disease by using the approaches described above. However, this approach is not useful for identifying novel disease-causing genetic changes in human cancers. To define novel disease-relevant activities, an unbiased approach needs to be applied. For example, specific RNA interference (RNAi) and shRNA libraries can be constructed in a manner that allows knockdown of the expression of genes encompassed in regions of recurrent genomic loss, promoter methylation or epigenetic silencing. Such an unbiased screen can functionally identify the relevant tumor suppressor activities affected by genomic changes in human cancer cells (Fig. 1). Alternatively, the results of genome-wide screens – for example, using libraries of cDNAs or shRNAs, or using viral and transposon insertion-site analyses – can be cross-compared to the results of genomic analyses of human tumors. In this manner, mouse models provide a functional filter and indicate key genes targeted in human cancer and, as a bonus, they provide us with genetically defined tumors for further analyses or in vivo treatment studies.

Examples of successful oncogenic studies

A ‘functional oncogenic’ study of liver cancer that used RNAi constructs to silence genes that were found to be commonly deleted in patients with liver cancer successfully identified cIAP1, XPO4, FGF6 and the RNA helicase DDX20, amongst others, as tumor suppressors in this type of cancer (Zender et al., 2006; Xue et al., 2008; Zender et al., 2008). Other successful studies have involved in vivo screens that were not directed by insight into genomic changes; for example, key genetic changes were identified in the Eμ-Myc model of Burkitt’s lymphoma by using viral insertions (van Lohuizen et al., 1991; Uren et al., 2008; Kool et al., 2010) or shRNA libraries (Bric et al., 2009). Using a similar approach, we recently identified miR-19 as a key oncogenic microRNA in T-ALL by using a microRNA-library approach. We then used a parallel in vitro shRNA screen to identify functionally important miR-19 target genes (Mavrakis and Wendel, 2010; Mavrakis et al., 2010). These studies indicate that the combination of microRNA- and shRNA-library screens can be used to identify oncogenic microRNAs and their relevant target genes. In ongoing studies, we are employing these approaches to functionally dissect genomic lesions such as loss of chromosome 6q, which occurs with frequencies of up to 30% in human Non-Hodgkin’s lymphoma.

Fig. 1. Mouse models as biological filters for large-scale tumor genome data. Genetic studies of highly versatile mouse models of hematopoietic cancers serve as biological filters for complex cancer genome data, and allow information about biological function and clinical significance of genetic driver mutations to be obtained. In this manner, mouse models can help to translate genomic data into better molecular diagnostics for personalized medicine. aCGH, array comparative genomic hybridization; SNP, single nucleotide polymorphism.
The above approaches are based on positive screens – that is, screens for genetic lesions that enhance tumor formation in a mouse model and the subsequent comparison of the results with data from human cancer genomes. A variation of this approach, referred to as an ‘Achilles heel screen’, is designed to identify genes whose inactivation cannot be tolerated by a tumor, making them potential drug targets. Such negative or ‘drop-out’ screens give insight into potential synthetic lethal relations and new treatment strategies. In addition, they reveal key activities involved in carcinogenesis and, again, comparing the results of such an unbiased screen with genomic analyses of human cancers can help to pinpoint key cancer genes. This approach has been pursued extensively in human cell lines that represent lymphoma or solid tumors (Lam et al., 2008; Schlabach et al., 2008; Shaffer et al., 2008; Silva et al., 2008; Bidere et al., 2009). More recently, this strategy has also been applied to genetically defined mouse lymphoma models (Meacham et al., 2009). Clearly, functional genetic screens can complement genomic tumor analyses and provide new insight into cancer-relevant activities.

**Pitfalls and potential solutions**

Comparing functional genetic studies and large-scale genetic screens with complex genomic data from human cancers can involve very complex experimentation. So, it is important to consider caveats of this approach and potential solutions. For example, library screens – both positive and negative screens – often yield false-positive or false-negative data. False-positive ‘hits’, which can be caused by random integrations, off-target effects or unphysiological expression levels of exogenous genes, can be eliminated in validation studies. False-negative data imply that genetic changes (e.g. shRNA vectors) that should have been identified in a screen were missed. This might reflect technical shortcomings or problems in experimental design. For example, current shRNA and RNAi libraries are not validated for target knockdown; therefore, it is possible that effective knockdown of a key gene is not achieved by the shRNA designed to target it. Furthermore, experimental conditions can limit the number of shRNAs that can be screened in parallel. This is especially true for in vivo screens that involve the adoptive transfer of hematopoietic progenitor cells. For example, the very small number of hematopoietic progenitors that actually contribute to the hematopoietic reconstitution represents a physiological ‘bottleneck’ and therefore limits the size of a pooled library that can be screened. Library reconstruction experiments test the ability to identify a positive control from increasing dilutions and allow an estimation of the size of library pools that can be screened in a given experiment. Typically, in vitro screens in homogenous cell populations permit testing of larger library pools. A pragmatic approach might be to perform a screen in vitro, followed by an in vivo validation step (Mavrakis and Wendel, 2010). Clearly, it is important to consider the experimental context, taking into account, for example, the origin of cells and their genetic background, because these factors all shape the results and interpretation of a screen. Although technically challenging, new technologies such as large-scale loss-of-function screens enable a new level of insight into cancer biology.

**Translating ‘filtered’ genomic data to the clinic**

Insights into the genetics of cancer will influence how these diseases are diagnosed and treated as we move from pathology-based towards molecular medicine. As discussed above, mouse models provide a filter that allows us to identify from genomic tumor studies those key genetic changes that drive tumor biology. It is most likely that these genetic changes are important determinants of tumor biology and that they underlie some of the heterogeneous responses to treatment that are observed among patients with pathologically identical tumors. We anticipate that genes that are involved in tumor evolution and targeted by genomic aberrations or epigenetic mechanisms can also serve as molecular markers that will enable us to better stratify patient populations and assign the most suitable treatment for individuals or groups of patients. It is likely that functional insight into tumor genetics will help to improve current treatments and lead to the identification of new targets for mechanism-based therapies.

**Outlook**

The development of new tools for the genomic analysis of human cancers, together with the functional genetic dissection of cancer genome data using mouse models, will lead to detailed insight into the molecular pathogenesis of cancer. We consider these as complementary approaches that can highlight genes and pathogenic mechanisms that would not emerge from the use of either approach alone. We have focused here on hematopoietic malignancies and tumor initiation (oncogenesis), but clearly the approaches discussed are more broadly applicable. For example, they could also be used to probe the molecular events that underlie problems such as drug resistance, disease relapse, or invasion and metastasis in the case of solid tumors. Furthermore, we expect that these approaches will be useful for uncovering genetic changes that do not behave as classical oncogenes or tumor suppressors but that modify tumor phenotypes in a measurable manner. Clinical studies will reveal whether such modifier genes produce significant effects in humans.

Ultimately, insights into tumor genetics should lead to new treatment strategies. Mouse models provide an opportunity to genetically inactivate potential drug targets – in the tumor or the whole animal – long before a pharmacological inhibitor of the target is available. At the clinical level, a move from pathology-based diagnoses towards molecular medicine is in progress, and we believe that this shift will be facilitated by the use of mouse models to prioritize candidate genes for biomarkers or therapeutic targets.

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**COMPETING INTERESTS**

The authors declare no competing interests.

**REFERENCES**


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