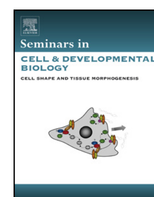
Seminars in Cell & Developmental Biology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb



Review

Mouse models of cancer: *Sleeping Beauty* transposons for insertional mutagenesis screens and reverse genetic studies

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A R T I C L E I N F O

Article history:
Available online xxx

Keywords:
Transposable elements
Sleeping Beauty
Insertional mutagenesis
Mouse models
Cancer gene discovery

A B S T R A C T

The genetic complexity and heterogeneity of cancer has posed a problem in designing rationally targeted therapies effective in a large proportion of human cancer. Genomic characterization of many cancer types has provided a staggering amount of data that needs to be interpreted to further our understanding of this disease. Forward genetic screening in mice using *Sleeping Beauty* (*SB*) based insertional mutagenesis is an effective method for candidate cancer gene discovery that can aid in distinguishing driver from passenger mutations in human cancer. This system has been adapted for unbiased screens to identify drivers of multiple cancer types. These screens have already identified hundreds of candidate cancer-promoting mutations. These can be used to develop new mouse models for further study, which may prove useful for therapeutic testing. *SB* technology may also hold the key for rapid generation of reverse genetic mouse models of cancer, and has already been used to model glioblastoma and liver cancer.
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1. Introduction

It is becoming increasingly clear that cancer is a very complex and heterogenous disease where each individual cancer type is actually composed of multiple molecular subclasses. Cancer arises from a series of genetic events that result in the corruption of normal cellular development, growth, and proliferation. A variety of sets of genetic events can corrupt these processes, which underlie the variety of molecular subclasses of cancer. In order to develop focused and effective means of treating the disease, greater research is required to further elucidate the cancer-promoting genes that contribute to these subclasses and determine how

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http://dx.doi.org/10.1016/j.semcdb.2014.01.006
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they function and cooperate in promoting tumorigenesis. Large-scale genomic characterization efforts by The Cancer Genome Atlas (TCGA) network and other groups are revealing staggering numbers of genes mutated, lost, amplified, or dysregulated in human cancer. Some of these alterations have already been identified as recurrent and shown to promote cancer phenotypes, providing insight into the mechanisms of cancer pathogenesis and potential therapeutic strategies, such as *EGFR* fusions and mutations promoting glioblastoma growth, which may inform the design of clinical trials for EGFR inhibitors for glioblastoma [1]. Unfortunately for many cancers, the confusing heterogeneity of underlying mutations leading to similar cancer phenotypes has precluded the ability to design targeted therapies that are effective in a large percentage of cancer patients. The amount of mutation information becoming available highlights the need for effective methods to distinguish between passenger alterations, which result from genomic instability and have no role in tumorigenesis, and driver alterations, which promote tumor progression and maintenance and, importantly, may serve as effective therapeutic targets or prognostic markers. Models that accurately reflect this genetic heterogeneity and allow it to be understood are desperately needed to identify driver mutations and design rational targeted therapies.

Unbiased screens for cancer promoting mutations provide a means of distinguishing driver from passenger mutations. In transposon-based mutagenesis screens, the random insertion of mutagenic transposons alters normal endogenous genes in the mouse and induces cancer. The genetic changes that drive disease progression can then be identified by the locations of transposon insertions [2–9]. These transposon-based systems, therefore, represent powerful genetic tools for identifying cancer-promoting mutations. This unbiased method of elucidating cancer genes has proven effective. Information derived from these screens and the resulting new cancer models based on this information will contribute greatly toward developing and testing effective therapeutic regimes. Importantly, transposons can be used as both forward and reverse genetic tools to elucidate cancer genes *in vivo*.

The *Sleeping Beauty* (*SB*) transposable element is a synthetic DNA-type transposon belonging to the Tc1/Mariner transposon family that mobilizes in a "cut-and-paste" fashion. It was awakened from millions of years of evolutionary sleep by correcting the mutations responsible for its transposase inactivity [10]. The current *SB* transposon system consists of two parts: firstly, a transposon vector containing any DNA sequence that is flanked by inverted repeat/direct terminal repeat (IR/DR) sequences and secondly, the *SB* transposase enzyme that is responsible for excision and reintegration of the transposon placed under the control of a promoter. When both these components are present in a cell, a "cut-and-paste" transposition reaction occurs in which the transposon is excised from its original location and re-integrated at a new location within the genome. The mobilization process is relatively random, although it has the propensity for "local hopping" and the only prerequisite that the transposon reintegrates at a "TA" dinucleotide [11]. *SB* transposition is active in both transgenic mouse germline and somatic cells [2,3,11,12]. The mutagenic transposon called T2/Onc (Fig. 1A) was designed to cause both gene loss- and gain-of-function insertional mutations, which would be marked by the unique transposon sequences and could be used later to identify cancer genes in solid tumors (Fig. 1B and C). T2/Onc combined with transgenes ubiquitously expressing *SB* transposase in wild-type or cancer predisposed mice induced or accelerated sarcoma and T-cell leukemia [2,3]. In both cases, the *SB*-initiated or accelerated tumors were characterized by somatic, tumor-specific transposon insertions that were shown to occur at dozens of recurrently mutated known and novel cancer genes [2,3]. *SB* insertion sites are readily cloned and can be characterized rapidly to implicate new genes in solid tumor development using a forward genetic approach. Next-generation sequencing platforms allow for rapid and adequate coverage to identify transposon insertion sites. Sites mutated by insertions significantly more frequently in tumors than predicted by random chance are called common insertion sites (CISs) and are hypothesized to reveal potential driver mutations. This data can be applied to human cancer mutation data to elucidate which alterations found in human tumors may be important cancer drivers (Fig. 2). Comparison between CISs from *SB* screens and human tumor mutations has already implicated many genes in human disease including colorectal cancer, pancreatic cancer, medulloblastoma, and malignant peripheral nerve sheath tumors [8,13–16].

Tissue-specific mutagenesis with *SB* has developed informative models of various types of human solid tumors. In these studies, mice express the catalytically improved *SB* transposase, SB11 [17], from the endogenous *Rosa26* locus, but only after Cre recombinase has excised a loxP-flanked stop cassette (lsl) separating the SB11 cDNA from the *Rosa26* promoter. Thus, using these *Rosa26*-lsl-SB11 mice, *SB* mutagenesis can be restricted to tissues expressing the Cre recombinase from a tissue-specific promoter. Conditional *SB* transposition systems have been successfully used to generate various solid tumors and screen for genes associated with these cancer types [4,5,9]. In addition to tissue-specific promoters driving Cre recombinase, predisposed genetic backgrounds can also be incorporated into these screens to elucidate mutations cooperating with common cancer initiating mutations [5,6]. Variations of this mutagenesis system have been used to reveal both known and novel oncogenes as well as tumor suppressors in solid tumors [2,4–9]. Conditional mutagenesis systems with hematopoietic cell-specific promoters driving Cre recombinase and predisposed genetic backgrounds have proven useful for modeling liquid tumors such as B-cell precursor acute lymphoblastic leukemia, T-cell acute lymphoblastic leukemia, chronic lymphocytic leukemia, and erythroleukemia [18–21]. This review, however, will focus on solid tumor models.

Cancer-promoting mutations discovered from body wide and tissue specific mutagenesis screens can be compared to human mutation, gene copy number, and gene expression data to identify key cancer-promoting alterations. These alterations may provide novel, effective therapeutic targets and prognostic markers that may inform treatments. In addition, they can be used to create new mouse models of cancer that accurately reflect the many molecular subclasses of the human disease, which could be used for testing novel therapies. Recently, much progress has been made in identifying candidate cancer drivers for many types of cancer using transposable elements [22]. To continue this progress, new mouse models driven by these genes must be developed. Engineered mouse models of several types of cancer including liver cancer [23–25], and neurofibroma/malignant peripheral nerve sheath tumor [26,27], and glioma [28] that may prove useful for therapeutic testing have been developed. The methods used to generate these models may inform the development of other useful cancer mouse models driven by the genes identified in forward genetic screens.

2. Mouse models of cancer

2.1. Forward genetic screens for gastrointestinal tract cancer genes

Colorectal carcinoma (CRC) is the third most commonly diagnosed cancer for both men and women in the United States, and, with approximately one-third of patients dying from this disease, is a leading cause of cancer-related death [29,30]. It develops through the accumulation of mutations in genes belonging to

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