A Modified *Sleeping Beauty* Transposon System That Can Be Used to Model a Wide Variety of Human Cancers in Mice

Adam J. Dupuy, Laura M. Rogers, Jinsil Kim, Kishore Nannapaneni, Timothy K. Starr, Pentao Liu, David A. Largaespada, Todd E. Scheetz, Nancy A. Jenkins, and Neal G. Copeland

Department of Anatomy and Cell Biology, Center for Bioinformatics, Computational Biology and Biomedical Engineering, and Ophthalmology and Visual Sciences, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, Iowa; Department of Genetics, Cell Biology, and Development, Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota; Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom; and Institute of Molecular and Cell Biology, Proteos, Singapore

Abstract

Recent advances in cancer therapeutics stress the need for a better understanding of the molecular mechanisms driving tumor formation. This can be accomplished by obtaining a more complete description of the genes that contribute to cancer. We previously described an approach using the *Sleeping Beauty* (SB) transposon system to model hematopoietic malignancies in mice. Here, we describe modifications of the SB system that provide additional flexibility in generating mouse models of cancer. First, we describe a Cre-inducible SBase allele, RosaSBase<sup>1,4</sup>, that allows the restriction of transposon mutagenesis to a specific tissue of interest. This allele was used to generate a model of germinal center B-cell lymphoma by activating SBase expression with an Aid-Cre allele. In a second approach, a novel transposon was generated, T2/Onc3, in which the CMV enhancer/chicken β-actin promoter drives oncogene expression. When combined with ubiquitous SBase expression, the T2/Onc3 transposon produced nearly 200 independent tumors of more than 20 different types in a cohort of 62 mice. Analysis of transposon insertion sites identified novel candidate genes, including Zmir1 and Rian, involved in squamous cell carcinoma and hepatocellular carcinoma, respectively. These novel alleles provide additional tools for the SB system and provide some insight into how this mutagenesis system can be manipulated to model cancer in mice. [Cancer Res 2009;69(20):8150–6]

Introduction

The relationship between somatic mutation and cancer is well established. We understand that the stepwise acquisition of mutations over time drives cancer progression. These ideas are well defined and provide a basic framework for the field of cancer genetics, but our ability to precisely identify the gene mutations that contribute to an individual tumor remains limited.

Recent efforts to resequence coding exons in human tumors have identified somatic mutations in breast and colorectal cancer (1), pancreatic cancer (2), glioblastoma multiforme (3), and lung cancer (4). These studies found an average between 47 and 80 coding region somatic mutations per tumor. One major implication of this work is that the number of “passenger mutations” greatly exceeds the number of “driver mutations.” Consequently, more sequencing will be required to gain a comprehensive view of the human cancer genome. This is especially true given the relatively low mutation rate of most cancer genes within a patient population (1).

We recently described a novel transposon-based insertional mutagenesis system, called *Sleeping Beauty* (SB), which can be used as a cancer gene discovery tool in mice (5, 6). Our initial version of the SB system consisted of the RosaSBase knock-in allele (transposase source) and the T2/Onc2 allele (transposase source). A significant number of mice that inherit both alleles die during embryogenesis, and the remaining mice develop aggressive tumors (for review, see refs. 7, 8). Mutations in SB-induced tumors are caused and tagged by transposon insertions, facilitating the identification of cancer genes.

The initial SB system had several significant limitations. First, the high rate of embryonic lethality limited the production of RosaSBase:T2/Onc2 double-transgenic mice. Second, the initial SB system produced hematopoietic malignancies, yet failed to produce carcinomas, which occur more frequently in the human population (6). These features limited the ability of the SB system to model cancer in mice.

Here, we describe a Cre-inducible SB transposase allele, in addition to a novel mutagenic transposon, T2/Onc3. These new components provide additional flexibility to the system, greatly broadening the tumor spectrum that can be produced by *in vivo* transposon mutagenesis.

Materials and Methods

Generation of RosaSBase<sup>1,4</sup> and T2/Onc3 mice. The RosaSBase<sup>1,4</sup> targeting construct was generated by cloning three polyadenylic acid [poly(A)] signals [two SV40 poly(A) sites and one bovine growth hormone poly(A)] downstream of a farnesylated enhanced green fluorescent protein (EGFPF) cDNA (Clontech). Flanking loxP and restriction sites were added to this amplified cassette to allow the fragment to be cloned between the En2 splice acceptor and SBase cDNA in the targeting construct used to produce the RosaSBase allele (5). A PGkneo cassette flanked by FRT sites was inserted downstream of the SBase-poly(A) sequences. The targeting vector was linearized and electroporated into hybrid C57BL/6J<sup>129</sup> ES cells. Following selection, clones were screened by Southern blotting. Chimeric offspring resulting from blastocyst injection were bred to C57BL/6J mice to establish the line.

The T2/Onc3 transposon was generated by replacing an *EcoRI/HindIII* fragment from the pT2/Onc2 plasmid (5) containing the murine stem cell virus (MSCV) 5′ long-terminal repeat (LTR) promoter with a *EcoRI/HindIII* fragment containing the chicken β-actin (CAG) promoter. The pT2/Onc3 plasmid was linearized by *SacI*. Linear plasmid DNA was injected into...
C57BL/6J × C3H hybrid one-cell embryos. Tail-biopsy DNA from resulting offspring were screened by Southern blotting. All animal experiments were approved and monitored by the National Cancer Institute-Frederick Animal Care and Use Committee.

**Immunohistochemistry and histopathology.** The Comparative Molecular Pathology Unit at National Cancer Institute-Frederick performed histopathology and tumor classification.

**Statistical analysis of common integration sites.** Monte Carlo and gene-based Monte Carlo methods are described in the Supplementary Methods.

**Paraffin block RNA extraction and reverse transcription-PCR to detect Zmiz1 fusion transcripts.** Total RNA was extracted from formalin-fixed, paraffin-embodied mouse tumor tissue using the RecoverAll Total Nucleic Acid Isolation Kit (Ambion). Reverse transcription-PCR was performed with the One-Step RT-PCR Kit (Qiagen). Primer sequences were as follows: SD-F (5'-AAGTCGTACTAGCACCAGAACGC-3'), SA-R (5'-GCTACGTTGCTAACAACCTGCGG-3'), ex8-F (5'-TTCTCTTATGACCTGCTCCCTTG-3'), ex9-R (5'-GGTGGTGGTCTAGCCTAGTG-3'), mGAPDH-F (5'-GGCTACGTTGCTAACAACCTGCGG-3'), and mGAPDH-R (5'-AATTGTGCGTCCGTGATGCT-3').

**Sequence analysis and annotation.** GS FLX sequences were analyzed to identify the expected transponson/adaptor sequences using BLAST (9). The flankering genomic insert was extracted into a separate file. Sequences with the correct structure were sorted via barcode incorporated during PCR. The junction sequence was aligned to the mouse genome (mm9) using BLAT (10). Unique best hits were identified from the genome alignments. In cases of multiple alignments, the score of the second-best alignment was required to be at least 5% lower than the score of the best alignment. Integrations were aggregated for each tumor and annotated based on the chromosome, basepair position, and the number of sequence reads from that integration site. The UCSC genome annotation database refFlat table was used to determine if integration occurred within, or nearby, a gene (11). If so, the gene symbol, relative location within the gene, expected effect on expression, and orientation with respect to the gene were annotated.

**Monte Carlo simulation to identify significant common integration sites.** A modified Monte Carlo method generated a randomly selected TA site in the mouse genome for each transposon integration that had been identified. Next, 1 × 10^6 iterations of the simulation were performed. The output was used to define a common integration site (CIS) as the minimum genomic region in which N different insertions are observed to be significant (P = 0.0001) when compared with 1 × 10^6 iterations of the Monte Carlo simulation. We determined this value for CISs that contain between 3 and 10 insertions and for which a single tumor contributes no more than 2 of the events (Supplementary Table S3).

### Results

**Cre-inducible SB mutagenesis.** By restricting transposase expression to a specific tissue, we hoped to reduce or eliminate embryonic lethality and generate tumors in any desired tissue concomitantly. We elected to use a lox-stop-lox approach, in which the RosaSBase allele is under the control of a floxed transcriptional stop cassette (Fig. 1A); an approach already used to produce many lox-stop-lox ROSA26 alleles which express a reporter in a Cre-dependent manner (12, 13).

We used a similar strategy to introduce a floxed stop cassette, consisting of an EGFP cDNA followed by three tandem poly(A) signals, upstream of the SBase cDNA in the RosaSBase allele, thereby producing the RosaSBase<sup>LS</sup> allele (Fig. 1A). This design should ensure that only EGFP is expressed prior to the removal of the lox-stop-lox cassette by Cre recombination. The RosaSBase<sup>LS</sup> cassette was targeted to the Gt(Rosa)26Sor locus in mouse ES cells, and Southern blotting of ES cell clones confirmed the structure of the RosaSBase<sup>LS</sup> allele (data not shown). Chimeric mice generated by blastocyst injection were bred to C57BL/6J mice to propagate the line.

Next, we tested the function of the RosaSBase<sup>LS</sup> allele. The lox-stop-lox cassette was excised in the germ line by crossing RosaSBase<sup>LS</sup>/<sup>LS</sup> mice to β-actin-Cre transgenic mice. This produced the RosaSBase<sup>HoxP</sup> allele, which differs from the RosaSBase allele in that a single loxP site lies between the splice acceptor and the SBase cDNA (Fig. 1A). We predicted that the RosaSBase<sup>HoxP</sup> allele would function in a similar fashion to RosaSBase. Thus, we expected that a significant number of RosaSBase<sup>HoxP</sup>/T2/Onc2 double-transgenic embryos would die during gestation, and that the remaining mice would develop aggressive hematopoietic tumors with a short latency.

To test this hypothesis, we generated a small cohort of animals (n = 24) by crossing heterozygous RosaSBase<sup>HoxP</sup> mice to TG6070 mice homozygous for a T2/Onc2 transposon concatenator array that contains ~200 copies of the transposon (6). Despite our prediction, we did not see evidence of embryonic lethality in this cohort (Supplementary Fig. S1). It is possible that the loxP site and additional sequences in RosaSBase<sup>HoxP</sup> interfere with the translation of the transcript. However, SBase protein levels in tissues from RosaSBase and RosaSBase<sup>HoxP</sup> mice were not dramatically different (Supplementary Fig. S1). We predicted that RosaSBase; TG6070 double transgenic mice display the least amount of embryonic lethality of the three T2/Onc2 strains previously described (6), and R. Shallow changes in the SBase expression level could reduce or eliminate the lethality. Despite the apparent difference between the RosaSBase and RosaSBase<sup>HoxP</sup> alleles, all RosaSBase<sup>HoxP</sup>; TG6070 double-transgenic mice developed hematopoietic tumors (Fig. 1B). Tumor latency (~ 59 days) is consistent with the results obtained using RosaSBase as the transposase source (6). Therefore, a reduction in transposition efficiency induced by the RosaSBase<sup>HoxP</sup> allele does not significantly affect tumor latency when combined with the TG6070 T2/Onc2 transposon donor transgene.

Finally, RosaSBase<sup>LS</sup> allele tissue specificity was tested by generating triple-transgenic mice that were heterozygous for RosaSBase<sup>LS</sup>, the T2/Onc2 transposon array, and an Aid-Cre knock-in allele. The Aid-Cre allele has a Cre recombinase cDNA fused to the initiation codon of the activation-induced cytidine deaminase gene (Aicda), and should drive Cre-mediated recombination in germinal center B-cells. Triple-transgenic mice and double-transgenic littermate controls that did not inherit the Aid-Cre allele were aged (Fig. 1B). A log-rank test showed that triple-transgenic mice had significantly decreased survival compared with their double-transgenic littermates (P = 0.0322; Fig. 1B). The pathology of moribund mice indicated that >90% of triple-transgenic mice developed tumors with an average latency of 46 weeks. In contrast, double-transgenic mice were not tumor prone, although many were euthanized due to complicating pathologies such as urinary tract infection (Supplementary Table S1). Similar pathologies were not noted in other RosaSBase<sup>LS</sup>; T2/Onc2 cohorts we generated (data not shown), or in another experiment using the RosaSBase<sup>LS</sup> allele (14). Thus, the complicating pathologies seen in double-transgenic control mice are not likely induced by transposition.

The majority of tumors developed by triple-transgenic mice were of B-cell origin, including diffuse large cell lymphomas and follicular lymphomas (Fig. 1C; Supplementary Table S1). RosaSBase<sup>LS</sup>; T2/Onc2 double-transgenic mice predominantly developed T-cell lymphomas, consistent with our previous findings (Fig. 1C; ref. 6). Notably, some of the triple-transgenic mice developed malignancies in other cell types (Fig. 1C).
Carcinomas induced by T2/Onc3 mutagenesis. We previously showed that ubiquitous transposition induces hematopoietic tumors in mice, although transposition could be detected in other cell types (6). Although many factors could contribute to bias in tumor spectrum, one that can be easily manipulated is transposon design. The T2/Onc2 transposon contains the 5' LTR from the MSCV to drive the overexpression of oncogenes (6). The MSCV is derived from a cloned Moloney murine leukemia virus (15), and is likely to drive oncogene expression in hematopoietic cells at a higher rate than in other cell types. We reasoned that swapping the MSCV LTR promoter for one that expresses strongly in epithelial cells should alter the tumor spectrum produced.

We generated a new mutagenic transposon, T2/Onc3, the structure of which is identical to that of T2/Onc2 (6) except that the MSCV LTR is replaced with the CAG promoter (Fig. 2A). This promoter consists of the cytomegalovirus immediate early enhancer fused to the CAG promoter, and expresses strongly in a variety of cell types in vivo, including epithelial cells (16, 17). We then tested the effect of this change on the tumor spectrum.

The T2/Onc3-containing plasmid was linearized and injected into C57BL6/J × C3H F1 one-cell embryos. Founder mice were initially identified by Southern blotting. Estimating transposon copy number is important, as this variable has been shown to have an effect on tumor latency (7). We were previously able to generate high-copy transposon donors (150–300 copies) using the T2/Onc2 transposon (6). However, we were unable to produce high-copy T2/Onc3 transgene founders, despite screening more than 340 founder animals (data not shown). We generated two T2/Onc3 transgenic lines from two founder animals (TG12740 and TG12775) by crossing them with wild-type C57BL6/J mice. Using quantitative PCR, we estimate that the TG12740 and TG12775 transgenes contain approximately 11 and 28 copies, respectively (Supplementary Fig. S2).

Heterozygous T2/Onc3 transgenic mice from each line were then crossed to homozygous RosaSBase animals to induce ubiquitous transposition. Double-transgenic T2/Onc2;RosaSBase<sup>loxP</sup> mice were also aged to determine tumor susceptibility. C, types and frequency of tumors for triple-transgenic mice (left) and double-transgenic T2/Onc2;RosaSBase<sup>loxP</sup> mice (right).
embryonic lethality (Supplementary Table S2). This was expected, because it was previously shown that the combination of RosaSBase and a low copy transposon donor does not cause embryonic lethality (7).

We generated and aged a cohort of 73 double-transgenic mice, along with 45 RosaSBase single-transgenic littermate controls. Compared with single-transgenic RosaSBase mice, double-transgenic mice had a decreased rate of survival (log rank, \( P < 0.0001; \) Fig. 2B). Histologic analysis was performed on all double-transgenic mice for which material was available (\( n = 62 \)) and on 19 randomly selected RosaSBase control mice (Table 1). In contrast with control mice, double-transgenic mice develop a variety of malignancies, with each animal developing three independent primary tumors on average. Only 11% of T2/Onc3-induced tumors were lymphomas, compared with \( \sim 90\% \) of T2/Onc2-induced tumors (6). Instead, double-transgenic mice developed a wide array of carcinomas, including squamous cell carcinoma of the skin and hepatocellular carcinoma (Table 1). Notably, a significant gender bias was associated with both hepatocellular carcinoma (\( P = 0.0052 \)) and liver adenoma (\( P = 0.00028 \)); male double-transgenic mice were significantly more susceptible to liver malignancies. This is similar to the gender bias seen in human hepatocellular carcinoma (18).

Histologic examination found four cases of metastasis in RosaSBase/T2/Onc3 double transgenic mice (Table 1). Metastasis was associated with a variety of tumor types: melanoma, hepatocellular carcinoma, hemangiosarcoma, and salivary gland carcinoma (Fig. 3; Supplementary Fig. S4). In the metastatic hepatocellular carcinoma, numerous macrometastases were detectable in the lungs (Fig. 3B). We generated transposon integration profiles for both the primary liver tumor and one of the large metastatic lesions from the lung using the ligation-mediated PCR (LM-PCR) strategy subsequently described (Supplementary Fig. S3). Two identical transposon integration events were identified in both tumors, suggesting that these tumors share a common origin (Supplementary Table S3). However, the majority of integration events identified in each tumor were unique events.

Identification of transposon-induced mutations. We used a modified LM-PCR method to produce bar-coded PCR products from SB-induced tumors that could be directly sequenced on the GS FLX System (14, 19). Briefly, an "amplicon sequencing" approach was used in which unique tags were added to the 5'-ends of the primers used during the final amplification step of the LM-PCR. Additionally, we added a 10-bp barcode (20) to the 5'-end of the transposon-specific primer, such that it would be sequenced just before the transposon tag (Supplementary Fig. S3). Therefore, it is possible to specifically amplify transposon junction fragments from SB-induced tumors using LM-PCR, pool the resulting products, and sequence them in a single run on the GS FLX System.

We obtained adequate genomic DNA samples from 17 skin (squamous cell carcinoma) and 11 liver (hepatocellular carcinoma) tumors from T2/Onc3 mice, and these samples were analyzed using LM-PCR followed by pyrosequencing. This approach generated 692 sequences on average for each tumor, with as few as 261, and as many as 1,273 sequences per tumor. Overall, this represents a 3-fold increase over the sequence coverage previously used to identify insertion sites in SB-induced tumors (6). We developed an automated process to trim, map, and annotate each sequence called the Integration Analysis System.7

We were able to identify an average of 166 unique integration sites in each SB-induced skin (Supplementary Table S4) and liver

Table 1. Pathology of T2/Onc3-induced tumors

<table>
<thead>
<tr>
<th>Site</th>
<th>Type</th>
<th>No.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Squamous cell carcinoma</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Melanoma</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Basal cell carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Liver</td>
<td>Hepatocellular carcinoma</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Hepatoblastoma</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Adenoma</td>
<td>58</td>
</tr>
<tr>
<td>Colon</td>
<td>Carcinoma</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Adenoma</td>
<td>1</td>
</tr>
<tr>
<td>Lung</td>
<td>Carcinoma</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Adenoma</td>
<td>21</td>
</tr>
<tr>
<td>Brain</td>
<td>Astrocytoma</td>
<td>1</td>
</tr>
<tr>
<td>Mammary gland</td>
<td>Carcinoma</td>
<td>5</td>
</tr>
<tr>
<td>Ovary</td>
<td>Adenocarcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Clitoral gland</td>
<td>Squamous cell carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>Carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Preputial gland</td>
<td>Carcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Parathyroid</td>
<td>Carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Nasal cavity</td>
<td>Carcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>Pheochromocytoma</td>
<td>1</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>T cell</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>B cell</td>
<td>5</td>
</tr>
<tr>
<td>Multiple</td>
<td>Sarcoma</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Hemangiosarcoma</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Hemangioma</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Adenoma (other)</td>
<td>23</td>
</tr>
</tbody>
</table>

*Number of tumors in 62 T2/Onc3RosaSBase mice. The number of metastatic tumors of each type is shown in parentheses.
tumor (Supplementary Table S5). Most of the mapped integration sites (50–80%) were represented by a single sequence. It is possible that these sequences result from subclonal transposition events in rare tumor cells. Alternatively, these single sequences could arise from the contamination of normal cells that also undergo transposition in these mice due to ubiquitous transposase expression. In most tumors, however, a small number of integration sites contributed the majority of sequences. For example, an average of 45% of the sequences from each tumor were derived from only 10% of the integration sites (Supplementary Fig. S5). The clonal nature of these sites suggests that they are the initiating transposon-induced mutations that gave rise to the tumor (Supplementary Tables S4 and S5).

Analysis of transposon integration site data in SB-induced skin and liver tumors. More than 1,800 and 3,000 integration sites were identified in SB-induced skin and liver tumors, respectively. We then defined CIS by determining if specific genomic regions were disrupted by transposon integration at a significantly higher rate than expected. Previous SB mutagenesis screens have shown that such regions often contain cancer genes (5, 6). However, previous retroviral insertional mutagenesis screens have provided different definitions of a CIS (21, 22), raising the question of how best to determine their significance.

Significant CIS detection was recently improved by using Monte Carlo methods to simulate random integration in the host genome (14, 19). The simulated integration pattern could then be used to calculate the significance of any candidate CIS by determining the probability that integrations could occur by chance within a given set of data. Other methods developed to analyze integration patterns in lymphomas induced by retroviral insertional mutagenesis have recently been developed (23). However, a Monte Carlo method better simulates the specific features of SB transposition. For instance, the target sequence for SB transposon integration is a TA dinucleotide. The Monte Carlo simulation assumes that transposons will be randomly distributed at TA dinucleotide sites throughout the genome. One exception to this assumption is needed to account for local hopping because transposons tend to integrate in regions physically linked to the donor transgene. We determined the local chromosomes for TG12740 (chr9) and TG12775 (chr12) by analyzing the distribution of transposon integration sites in tumors (Supplementary Fig. S6). All integration events on the local chromosome were removed from the data set for each strain to eliminate false-positive CISs due to local hopping. Significant CISs were then identified using criteria defined by Monte Carlo simulation (Supplementary Table S6). This method identified three CISs in skin and two CISs in liver tumors (Table 2).

The most common transposon-induced mutation in liver tumors was within the Rian locus, and integrations were distributed throughout the 5′-end of the gene with no apparent bias for transposon orientation (Fig. 4A). The ProTIS analysis tool indicates that these integration sites are not predicated to be preferred TA sites (Supplementary Fig. S7; ref. 24). These observations strongly suggest that transposon disruption of the Rian gene is selected during T2/Onc3-induced hepatocellular carcinoma. The Rian locus is complex and includes three known micro-RNAs and a C/D

<table>
<thead>
<tr>
<th>Table 2. Identification of CISs using Monte Carlo simulation (α = 0.001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene symbol</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Zmiz1</td>
</tr>
<tr>
<td>Ppp1r3c</td>
</tr>
<tr>
<td>Orb270</td>
</tr>
<tr>
<td>Rian</td>
</tr>
</tbody>
</table>

Abbreviation: MIZ, MSX-interacting zinc finger.
snoRNA cluster (25). Additional experiments are therefore required to determine how the expression of these various elements is affected by transposon insertion.

More than 80% of SB-induced skin tumors had a transposon insertion in the Zmiz1 gene. Zmiz1 encodes a 115 kDa protein that contains a MSX-interacting zinc finger domain along with a putative nuclear localization signal and transcriptional activation domains (26). All 15 transposon integration events occurred in four different TA sites within intron 8 of Zmiz1 (Fig. 4B). Southern blot analysis confirmed the LM-PCR results in all cases, and identified additional SB-induced skin tumors with Zmiz1 integrations not analyzed by LM-PCR (Supplementary Fig. S8). All 15 integrations are in the same transcriptional orientation as Zmiz1 and are predicted to overexpress a fusion transcript between the CAG promoter in the T2/Onc3 transposon and exons 9 to 24 of Zmiz1. We confirmed the presence of a fusion transcript by reverse transcription-PCR (Fig. 4C). The fusion transcript is predicted to encode an NH2-terminally truncated protein. It is unclear how this truncation might affect Zmiz1 protein activity, as this NH2-terminal region does not contain any known functional motifs.

**Discussion**

In this report, we describe the generation of a Cre-inducible SB transposon system that eliminates the embryonic lethality associated with the original SB system (6), and allows tissue-specific control of SB transposase expression, modifying the tumor spectra produced. In the absence of a Cre transgene, the SB transposase is not expressed and tumors are not generated. This allows us to maintain mouse strains that are double homozygous for the conditional RosaSBaseLsL allele and a mutagenic transposon donor transgene. These mice can be used to generate large cohorts of tumor-prone animals through a single cross to a tissue-specific Cre transgenic line, and have already been used to generate models of colorectal cancer and hepatocellular carcinoma (14, 19). Here, we use an Aid-Cre allele to activate transposon mutagenesis in germinal center B-cells.

Finally, we show that altering the structure of the mutagenic SB transposon could have a profound effect on the tumor types induced by transposition. Although both are ubiquitous promoters, MSCV is very efficient in hematopoietic cells and CAG works efficiently in epithelial cells (27). By replacing the MSCV LTR in the original T2/Onc2 transposon with the CAG promoter, we shifted...
the tumor spectrum from mostly lymphomas to primarily carcinomas. This suggests that the promoter used to generate gain-of-function mutations in oncogenes upon SB integration significantly affects the tumor types produced. This further implies that the tumor spectrum generated by SB mutagenesis could potentially be altered by placing tissue-specific promoters within the mutagenic transposon. When combined with the Cre-inducible RosaSB base-allele, transposon promoter variants could provide even finer control of SB mutagenesis in vivo.

The SB-induced carcinoma model described is highly significant, not only because of its ability to generate solid tumors, but also because of its ability to produce metastatic tumors. Although the metastasis rate was low, it should be noted that each animal had an average of three independent tumors at the time of sacrifice, and high tumor burden may have caused the animals to become moribund before any macroscopic metastatic lesions could develop. Restricting SB mutagenesis to a specific tissue using the conditional RosaSB \textsuperscript{1,3} allele could potentially increase the rate of metastasis. An alternative approach would be to induce SB mutagenesis in a transgenic or knockout strain background that develops spontaneous tumors with low metastatic potential, in order to identify mutations that accelerate metastasis. The SB system could then be used to study metastasis by identifying genes that contribute to ability of a metastatic cell to survive and proliferate outside of the primary tumor site (28).

The power of forward genetic screens using SB transposition is greatly enhanced by larger tumor panels. Unfortunately, the types of carcinoma that developed in mice with the transposon carrying the CAG promoter were so diverse that we were unable to obtain significant numbers of tumors for most tumor types. However, we were able to identify novel candidate cancer genes for squamous cell carcinoma of the skin and hepatocellular carcinoma, the two most common tumor types in these mice. The limited number of samples is likely the reason we did not identify more candidate cancer genes in these two tumor types. The genomic landscapes of SB-induced tumors and human tumors are similar in that relatively few candidate cancer genes are mutated by SB transposon insertion in a large percentage of tumors. These genes can be easily identified even in small tumor panels (i.e., \textit{Zmiz1} and \textit{Rian}). Analysis of large SB-induced tumor panels will be required to identify candidate cancer genes that appear as gene hills on the cancer genomic landscape.

The SB system we describe here shows the flexibility and power of this system for identifying novel cancer genes in many tumor types. Our work also suggests that SB transposition is able to model many forms of human cancer in mice, including carcinomas. Thus, the SB system offers a complementary approach in the identification of cancer genes and may play an important role in deciphering the genetic complexity of human tumors. The SB system will likely identify a set of candidate genes that partially overlaps those identified in human tumors. Genes identified by both approaches are much more likely to represent cancer genes and would merit further study. Additionally, the SB transposition system could be used to address basic biological questions, such as metastasis or resistance to therapeutic agents, which would be difficult to address directly in human cancer patients.

Disclosure of Potential Conflicts of Interest

D.A. Largaespada: ownership interest, Discovery Genomics. The other authors declared no potential conflicts of interest.

Acknowledgments

Received 3/26/09; revised 7/17/09; accepted 8/18/09; published OnlineFirst 10/6/09.

Grant support: The production and characterization of all mouse strains was supported by the Department of Health and Human Services, NIH, and the National Cancer Institute. The molecular characterization of tumors was supported by a grant from the American Cancer Society (IGB-77-004-28).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank D. Swing and R. Koogle for generating the T2/Onc3 transgenic mice and maintaining all mouse strains, and E. Soutophon and S. Reed for generating mice carrying the RosaSB \textsuperscript{1,3} knock-in allele.

References

A Modified *Sleeping Beauty* Transposon System That Can Be Used to Model a Wide Variety of Human Cancers in Mice

Adam J. Dupuy, Laura M. Rogers, Jinsil Kim, et al.


**Updated version**
Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-09-1135

**Supplementary Material**
Access the most recent supplemental material at: http://cancerres.aacrjournals.org/content/suppl/2009/10/01/0008-5472.CAN-09-1135.DC1

**Cited articles**
This article cites 29 articles, 9 of which you can access for free at: http://cancerres.aacrjournals.org/content/69/20/8150.full#ref-list-1

**Citing articles**
This article has been cited by 33 HighWire-hosted articles. Access the articles at: http://cancerres.aacrjournals.org/content/69/20/8150.full#related-urls

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.