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***Sleeping Beauty* transposon insertional mutagenesis based mouse models for cancer gene discovery**

Branden S Moriarity^{1,2,3} and David A Largaespada^{1,2,3,4}

¹Department of Pediatrics, University of Minnesota Minneapolis, MN 55455, United States

²Center for Genome Engineering, University of Minnesota Minneapolis, MN 55455, United States

³Masonic Cancer Center, University of Minnesota Minneapolis, MN 55455, United States

⁴Department of Genetics, Cell Biology, and Development, University of Minnesota Minneapolis, MN 55455, United States

Abstract

Large-scale genomic efforts to study human cancer, such as the cancer gene atlas (TCGA), have identified numerous cancer drivers in a wide variety of tumor types. However, there are limitations to this approach, the mutations and expression or copy number changes that are identified are not always clearly functionally relevant, and only annotated genes and genetic elements are thoroughly queried. The use of complimentary, nonbiased, functional approaches to identify drivers of cancer development and progression is ideal to maximize the rate at which cancer discoveries are achieved. One such approach that has been successful is the use of the *Sleeping Beauty* (*SB*) transposon-based mutagenesis system in mice. This system uses a conditionally expressed transposase and mutagenic transposon allele to target mutagenesis to somatic cells of a given tissue in mice to cause random mutations leading to tumor development. Analysis of tumors for transposon common insertion sites (CIS) identifies candidate cancer genes specific to that tumor type. While similar screens have been performed in mice with the *PiggyBac* (PB) transposon and viral approaches, we limit extensive discussion to *SB*. Here we discuss the basic structure of these screens, screens that have been performed, methods used to identify CIS.

Development of *Sleeping Beauty* (*SB*) and general characteristics

SB is a two-component system comprised of a DNA element termed the *transposon vector* and an enzyme component, termed the *transposase*, originally constructed in Ivics *et al.* [1•]. When these two components are present within the same cell, the transposase recognizes the inverted repeat/direct repeat (IR/DR) sequences flanking the transposon and catalyzes the “cut-and-paste” reaction. In this process, the mobilized transposon vector is excised from the donor locus and reintegrated elsewhere at a TA dinucleotide. Several improvements to the *SB* system were made early on, including changes to the inverted terminal repeats that increase transposition rates [2]. Secondly, various catalytically improved versions of the *SB* transposase have been produced by site directed mutagenesis.

Because the first active version of the *SB* transposase was called SB10 [1^{••}], improved versions were called SB11 [3] and the like. By far the most active version is called SB100, and was the result of extensive screening of randomly generated derivatives of earlier versions [4].

Early studies using *SB* for germline mutagenesis and transgenesis identified important features of *SB* transposition that have relevance for somatic cell *SB* transposon mutagenesis. It was found transposition rates from multicopy transposon concatemers far exceeds the rate at which single-copy transposon vectors can be mobilized, even accounting simply for the increase in the number of substrates for transposition [5–7]. The reasons for this are not entirely clear, but it is known methylated *SB* transposon vector DNA is a better substrate for transposition compared to unmethylated transposon [8], and many transgene concatemers are partially methylated. A second important feature is called “local hopping.” Local hopping refers to the tendency of transposons to land near the donor concatemer on the same chromosome, usually within ~2–10 megabase pairs (Mb). This tendency is clear with *SB* transposition from donor concatemers located within mouse chromosomes for both germline and somatic cell transposition [5,7,9^{••},10^{••}].

***SB* mediated insertional mutagenesis for cancer gene discovery**

The *SB* system was first used for mutagenesis via a body wide screen for cancer in mice expressing the *SB* transposase (SB10 or SB11) and a mutagenic transposon line (T2/Onc or T2/Onc2) [9^{••},10^{••}]. Tumors resulted from insertion mutations in or near endogenous genes. In this work, loci recurrently mutated by transposons more than expected by chance, (that is, in multiple independent tumors) called common insertion sites (CIS), were identified. The T2/Onc transposons were designed to induce either gain-of-function (GOF) or loss-of-function (LOF) mutations when inserted in or near a gene based on its genetic cargo. The murine stem cell virus (MSCV) long terminal repeat (LTR) promoter with artificial exon and splice donor (SD) was included so downstream exons could be ectopically overexpressed as a consequence of fusion with transcripts initiated by the LTR and splicing from the T2/Onc SD. Many examples of ectopic overexpression of proto-oncogenes via this mechanism have now been described. In some cases, the fusion transcript produced encodes a full length protein as the targeted gene encodes a protein which has a translational start site in exon 2, for example in the case of activation of *Rspo2* [11^{••},12]. In other cases, insertion within a gene is followed by production of LTR initiated transcripts, splicing from the SD, to make fusion transcripts that encodes an N-terminally truncated protein translated from an internal ATG start codon, such as seen with *Notch1* [10^{••}] and *Braf* [9^{••}]. The T2/Onc vectors also included splice acceptors in both orientations and a bidirectional polyadenylation signal, to terminate transcripts that splice into the vector after insertion within an intron of a gene. In this way, many tumor suppressor genes (TSGs) have been inactivated as a consequence of *SB* insertional mutagenesis in various screens. Transcript termination has also resulted in proto-oncogene activation by C-terminal truncation, as in the case of *Egfr* [13^{••}]. Production of competitive endogenous RNAs (ceRNA), resulting in TSG downregulation via microRNAs, has also been observed as a consequence of *SB* transposon insertion [14].

One important variation to T2/Onc structure was made in version 3, termed T2/Onc3, in which the MSCV LTR was replaced by the CMV enhancer/chicken beta-actin (CAG) promoter [15•]. CAG has reduced activity in hematopoietic cell types and enhanced activity in epithelial cell types [15•]. Evidence suggests that T2/Onc3 may more potently induce tumors in epithelial tissues compared to T2/Onc and T2/Onc2, and lead to activation of proto-oncogenes more readily. The development of T2/Onc3 demonstrates that changes to the structure of *SB* transposons used for mutagenesis could reveal new kinds of genes and genetic elements in cancer development than have been discovered in screens so far.

It is important to consider the likelihood that *SB*-induced or accelerated tumors in mice have mutations caused by mechanisms other than T2/Onc insertional mutagenesis. Indeed, copy number alterations have been observed in tumor cells from *SB* cancer models [16•,17]. Nevertheless, *SB*-induced sarcomas generally have greatly reduced whole chromosome and gene copy number changes compared to sarcomas induced in mice without *SB* mutagenesis [16•]. The extent to which noninsertional mutagenesis mechanisms, such as point mutations, translocations, deletions and amplifications, contribute to *SB* models of cancer in mice remains to be determined.

The first *SB* mutagenesis studies generated both blood and solid cancers and identified CIS-associated genes [9•,10•]. The logical progression of the approach led to the development of a conditional *SB* transposase allele (Rosa26-LSL-SB11) that could be activated in a desired tissue when combined with tissue specific Cre recombinase transgene [11•,13•,15•]. This allows for tissue specific *SB* mutagenesis. The overall structure of most *SB* screens is shown in Figure 1. Since its inception the conditional *SB* transposon mutagenesis system has been applied to many cancer types, identifying hundreds of candidate cancer genes, generating new cancer models, and providing insights into the genes and mechanisms of cancer progression.

***SB* screens carried out so far**

To date there have been dozens of *SB* screens performed for various cancers on many different predisposing backgrounds, which have identified numerous known and novel cancer genes (Table 1). These data demonstrate many different tissues are amenable to *SB* mutagenesis. These include tissues derived from all three germ layers, resulting in carcinomas, sarcomas, neuroectodermal tumors, as well as hematopoietic cancers. Many of the most common and most deadly forms of human cancer have been modeled using *SB* mutagenesis.

We have learned from our own experiences, however, that not all cell types subjected to *SB* mutagenesis are equally amenable to tumor induction. In some of our work, the yield of tumors is low and latency long (data not shown). Thus we, and others, often use cancer prone genetic backgrounds in order to increase the tumor penetrance and decrease tumor latency. Tissue specific expression of oncogenes, such as *Kras*^{G12D} [18] and high level *EGFR* expression [17], and tissue specific or whole body TSG mutations, e.g. *Ptch* [19•] and *Trp53* mutations [17], have been widely implemented. In many or perhaps most cases, *SB* induced acceleration of cancer on a predisposed background is the only method to obtain a

high yield of tumors that develop within a reasonable length of time. Thus, care must be taken to choose the right version of the mutagenic *SB* transposon, Cre transgene, and cancer predisposed background (Figure 1). In any case, many reported models demonstrate the full spectrum of cancer development, including early benign lesions and malignant derivatives, including invasive and metastatic disease [16,20].

Interestingly, there is a large difference in the frequency at which candidate oncogenes are identified compared to TSG between screens that modeled solid or hematopoietic cancer types. The frequency of oncogene candidates identified in hematopoietic cancers is much higher than observed in solid tumor screens, in which TSG candidates dominate. This is probably because most screens performed to date have used T2/Onc or T2/Onc2, which harbor the MSCV LTR, capable of efficient proto-oncogene activation in hematopoietic cells, but less so in other cell types. Thus, use of T2/Onc or T2/Onc2 lines may favor TSG inactivation as a mechanism of tumor induction during solid tumor development. Use of T2/Onc3 in more tissues may reveal additional proto-oncogenes in new screens. However, it is clear T2/Onc3 also disrupts TSG in screens reported so far [15].

Current and future refinements of the approach

Several improvements, new methods of analysis, and applications of *in vivo*, somatic cell, transposon-based, insertional mutagenesis can now be envisaged or are underway (Figure 2). Indeed, the long and storied history of forward genetic screening in model organisms is marked by innovation in approaches and more and more complex screens. While initial *SB* work simply screened for cancer drivers in wild type mice, this was soon followed by screens for cancer genes and genetic pathways that cooperate with specific predisposing mutations [21,22]. Screens for specific cancer relevant traits, such as therapy resistance or metastasis could be very revealing. Indeed, several *SB* cancer models demonstrate overt metastasis [13,16] or invasive behavior [20] in a subset of cases and these studies have already revealed candidate genes whose alterations may cause these phenotypes. A recent study also showed that *SB* could be used to discover mediators of *Braf* inhibitor resistance in a model of melanoma in mice [23]. In a broader sense, *SB* models of cancer provide an opportunity to more realistically approximate a set of cancer cases like those that confront physicians in human patients. *SB* models provide a set of tumors with overlapping but highly complex mutations, which are tractable by analyses of insertion mutations. They also have intratumoral genetic heterogeneity, due to ongoing *SB* transposon mobilization as the tumor develops, a phenomenon that has been demonstrated in many types of human cancer [24]. These features could be used to help define genotype-phenotype correlations using *SB* models. The results would be especially relevant if these mouse models could be treated using therapies that mimic those given to human patients. In such a scenario, perhaps cancer cell autonomous determinants of treatment outcome could be discovered.

Other methodological approaches to analyze *SB* mutagenized tumors should also be considered. For example, most *SB* screens use ligation-mediated polymerase chain reaction (LM-PCR) based recovery of transposon insertion sites from tumor DNA [25]. However, almost all screens to date use restriction enzymes as a first step in the LM-PCR protocol. This leads to a bias in the recovery of specific insertions due to uneven distribution of such

sites. In fact, it has been demonstrated that shearing genomic DNA, before LM-PCR, allows for better recovery of insertions and leads to a good correlation between the number of times a given insertion is sequenced and the clonal abundance of cells with that insertion [26]. Thus, using this approach for insertion site recovery one could determine the “trunk” mutations that probably occurred very early in tumor development, versus the “branch” mutations that probably occurred later in tumor progression. Potentially, a different set of genes is characteristic for each kind of mutation. Another useful technology for such analyses would be the ability to recover transposon insertions from single cells. Generation of cell lines from *SB* induced tumors may allow specific phenotypes to be correlated with genotype more easily also.

Many *SB* screens are performed with two different T2/Onc (or T2/Onc2 or T2/Onc3) transgenic lines. In this way, it is possible to disregard local hopping and consider only insertion sites not linked to the donor concatemer but still cover the entire genome. To avoid using multiple concatemer lines, methods to eliminate local hopping in *SB* screens could be tried, such as addition of an artificial chromosome bearing the concatemer, or introduction of *SB* transposon vectors from plasmids, viral vectors, or episomal vector. Such approaches may have some practical value.

It is highly probable that complementary genomic analyses of *SB* induced tumors will be valuable. For example, it seems probable that some spontaneous genetic alterations cooperate with transposon insertion mutations in the development of these tumors. Therefore, array comparative genome hybridization, exome or whole genome sequencing and RNA sequencing may be revealing. RNA sequencing could be especially useful, because not only would the tumor gene expression profile (GEP) be revealed, but fusion transcripts between T2/Onc sequences (SA and SD) and endogenous transcripts would also be revealed. This would allow a focus on the most clonally abundant insertions, more easily determine whether a specific insertion was generating an LOF or GOF effect, and determine the target gene affected in the case of insertions within tightly clustered genes or for insertions some distance from their target gene.

Regardless of the details used to finally define all the CIS-associated genes, once a list of candidates is generated it can be used as an enriched list of tumor specific cancer genes for bioinformatic and comparative genomics analyses. For example, data from The Cancer Genome Atlas (TCGA), and similar projects, are becoming very valuable for comparing the specific genes and genetic pathways altered in both the *SB* mouse model and the corresponding form of human cancer [11•,27]. Such overlap provides strong evidence that a given gene or genetic pathway alteration is truly a cancer driver. However, functional validation in additional mouse models or human cells remains vital for making these determinations.

Concluding remarks

The development of *SB* and its use for cancer gene discovery has been a boon for cancer functional genomics studies. It has led to the discovery of new and specific cancer drivers critical to human tumor development or maintenance such as *Rspo2*, *Foxr2*, *Sema4d*, and

others. The approach has also uncovered roles for specific cancer pathways in human cancer such as Pten and Wnt/beta-catenin regulated pathways in malignant peripheral nerve sheath tumors. However, the full impact of the technology on our understanding of cancer has yet to be realized. This is in part because the specific genes and pathways discovered are so numerous that the rest of the field has not yet focused attention on them. A second reason, is that the general approach has not been used to study specific cancer relevant phenotypes or for enhancer/suppressor screens that would give specific molecular insight into a given pathway or biological process. If adapted to more sophisticated screens of this kind *SB* has the potential to allow insight into the behaviors of rare, yet critical cancer cells, such as metastatic or treatment resistant cells. Moreover, it could be used to dissect in greater detail, pathways under selective pressure for change, in the unique *in vivo* environment of a normal tissue, a growing tumor, and in a metastatic niche.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Ivics Z, Hackett PB, Plasterk RH, Izsvak Z. Molecular reconstruction of Sleeping Beauty, a Tc1-like transposon from fish, and its transposition in human cells. *Cell*. 1997; 91:501–510. [PubMed: 9390559] This paper describes the molecular reconstruction of SB for the first time and is the landmark paper in this field.
2. Cui Z, Geurts AM, Liu G, Kaufman CD, Hackett PB. Structure-function analysis of the inverted terminal repeats of the sleeping beauty transposon. *J Mol Biol*. 2002; 318:1221–1235. [PubMed: 12083513]
3. Geurts AM, Yang Y, Clark KJ, Liu G, Cui Z, Dupuy AJ, Bell JB, Largaespada DA, Hackett PB. Gene transfer into genomes of human cells by the sleeping beauty transposon system. *Mol Ther*. 2003; 8:108–117. [PubMed: 12842434]
4. Mates L, Chuah MK, Belay E, Jerchow B, Manoj N, Acosta-Sanchez A, Grzela DP, Schmitt A, Becker K, Matrai J, et al. Molecular evolution of a novel hyperactive Sleeping Beauty transposase enables robust stable gene transfer in vertebrates. *Nat Genet*. 2009; 41:753–761. [PubMed: 19412179]
5. Horie K, Kuroiwa A, Ikawa M, Okabe M, Kondoh G, Matsuda Y, Takeda J. Efficient chromosomal transposition of a Tc1/mariner-like transposon Sleeping Beauty in mice. *Proc Natl Acad Sci U S A*. 2001; 98:9191–9196. [PubMed: 11481482]
6. Horie K, Yusa K, Yae K, Odajima J, Fischer SE, Keng VW, Hayakawa T, Mizuno S, Kondoh G, Ijiri T, et al. Characterization of Sleeping Beauty transposition and its application to genetic screening in mice. *Mol Cell Biol*. 2003; 23:9189–9207. [PubMed: 14645530]
7. Dupuy AJ, Fritz S, Largaespada DA. Transposition and gene disruption in the male germline of the mouse. *Genesis*. 2001; 30:82–88. [PubMed: 11416868]
8. Yusa K, Takeda J, Horie K. Enhancement of Sleeping Beauty transposition by CpG methylation: possible role of heterochromatin formation. *Mol Cell Biol*. 2004; 24:4004–4018. [PubMed: 15082793]
9. Collier LS, Carlson CM, Ravimohan S, Dupuy AJ, Largaespada DA. Cancer gene discovery in solid tumours using transposon-based somatic mutagenesis in the mouse. *Nature*. 2005; 436:272–276. [PubMed: 16015333] This reference represents one of two studies published back-to-back demonstrating the use of Sleeping Beauty mutagenesis in mouse somatic cells for cancer gene discovery. T2/Onc and a CAGG-SB10 transgenic were utilized for body wide mutagenesis on a *Arf*^{-/-} predisposing background.

10. Dupuy AJ, Akagi K, Largaespada DA, Copeland NG, Jenkins NA. Mammalian mutagenesis using a highly mobile somatic Sleeping Beauty transposon system. *Nature*. 2005; 436:221–226. [PubMed: 16015321] This reference represents one of two studies published back-to-back demonstrating the use of Sleeping Beauty mutagenesis in mouse somatic cells for cancer gene discovery. T2/Onc2 and a Rosa26 knock-in of SB11 were utilized for body wide mutagenesis in otherwise wild-type mice.
11. Starr TK, Allaei R, Silverstein KA, Staggs RA, Sarver AL, Bergemann TL, Gupta M, O'Sullivan MG, Matise I, Dupuy AJ, et al. A transposon-based genetic screen in mice identifies genes altered in colorectal cancer. *Science*. 2009; 323:1747–1750. [PubMed: 19251594] This reference is one of the two first Sleeping Beauty forward genetic screens to utilize the Rosa26-LSL-SB11 conditional transgenic to perform tissue specific mutagenesis. Villin-Cre transgenic mice were utilized to activate SB11 in epithelial cells of the gastrointestinal tract to induce development of colorectal cancer in otherwise wild-type mice.
12. Seshagiri S, Stawiski EW, Durinck S, Modrusan Z, Storm EE, Conboy CB, Chaudhuri S, Guan Y, Janakiraman V, Jaiswal BS, et al. Recurrent R-spondin fusions in colon cancer. *Nature*. 2012
13. Keng VW, Villanueva A, Chiang DY, Dupuy AJ, Ryan BJ, Matise I, Silverstein KA, Sarver A, Starr TK, Akagi K, et al. A conditional transposon-based insertional mutagenesis screen for genes associated with mouse hepatocellular carcinoma. *Nat Biotechnol*. 2009; 27:264–274. [PubMed: 19234449] This reference is one of the two first Sleeping Beauty forward genetic screens to utilize the Rosa26-LSL-SB11 conditional transgenic to perform tissue specific mutagenesis. Albumin-Cre was utilized to activate SB11 in hepatocytes, leading to development of hepatocellular carcinoma on a wild-type and Trp53 deficient predisposing background.
14. Karreth FA, Tay Y, Perna D, Ala U, Tan SM, Rust AG, DeNicola G, Webster KA, Weiss D, Perez-Mancera PA, et al. *In vivo* identification of tumor-suppressive PTEN ceRNAs in an oncogenic BRAF-induced mouse model of melanoma. *Cell*. 2011; 147:382–395. [PubMed: 22000016]
15. Dupuy AJ, Rogers LM, Kim J, Nannapaneni K, Starr TK, Liu P, Largaespada DA, Scheetz TE, Jenkins NA, Copeland NG. A modified sleeping beauty transposon system that can be used to model a wide variety of human cancers in mice. *Cancer Res*. 2009; 69:8150–8156. [PubMed: 19808965] This reference demonstrated replacement of the MSCV promoter of T2/Onc and T2/Onc2 with the CAGG promoter shifts the tumor spectrum from largely hematopoietic cancer to more solid tumor development when body-wide mutagenesis is performed.
16. Moriarity BS, Otto GM, Rahrmann EP, Rathe SK, Wolf NK, Weg MT, Manlove LA, LaRue RS, Temiz NA, Molyneux SD, et al. A Sleeping Beauty forward genetic screen identifies new genes and pathways driving osteosarcoma development and metastasis. *Nat Genet*. 2015; 47:615–624. <http://dx.doi.org/10.1038/ng.3293>. [PubMed: 25961939] This reference utilized the Rosa26-LSL-SB11 transgenic in conjunction with Osterix1-Cre to induce osteosarcoma development and metastasis. Analysis of T2/Onc insertions between tumors and metastases demonstrated three distinct groups of relatedness among tumors and metastasis representing different models of metastasis development in osteosarcoma, i.e. parallel evolution, early dissemination, or a mixture thereof.
17. Rahrmann EP, Watson AL, Keng VW, Choi K, Moriarity BS, Beckmann DA, Wolf NK, Sarver A, Collins MH, Moertel CL, et al. Forward genetic screen for malignant peripheral nerve sheath tumor formation identifies new genes and pathways driving tumorigenesis. *Nat Genet*. 2013; 45:756–766. [PubMed: 23685747]
18. Perez-Mancera PA, Rust AG, van der Weyden L, Kristiansen G, Li A, Sarver AL, Silverstein KA, Grutzmann R, Aust D, Rummele P, et al. The deubiquitinase USP9X suppresses pancreatic ductal adenocarcinoma. *Nature*. 2012; 486:266–270. [PubMed: 22699621]
19. Wu X, Northcott PA, Dubuc A, Dupuy AJ, Shih DJ, Witt H, Croul S, Bouffet E, Fults DW, Eberhart CG, et al. Clonal selection drives genetic divergence of metastatic medulloblastoma. *Nature*. 2012; 482:529–533. [PubMed: 22343890] This reference utilized a Math1-SB11 transgenic to induce medulloblastoma development and metastasis on a Ptch or Trp53 deficient predisposing background. Analysis of T2/Onc insertions demonstrated the metastatic events took place early in tumor development as the tumor and metastases shared few T2/Onc insertions, demonstrating they may need to be treated as separate diseases in humans.

20. Takeda H, Wei Z, Koso H, Rust AG, Yew CC, Mann MB, Ward JM, Adams DJ, Copeland NG, Jenkins NA. Transposon mutagenesis identifies genes and evolutionary forces driving gastrointestinal tract tumor progression. *Nat Genet.* 2015; 47:142–150. [PubMed: 25559195]
21. Starr TK, Scott PM, Marsh BM, Zhao L, Than BL, O'Sullivan MG, Sarver AL, Dupuy AJ, Largaespada DA, Cormier RT. A Sleeping Beauty transposon-mediated screen identifies murine susceptibility genes for adenomatous polyposis coli (Apc)-dependent intestinal tumorigenesis. *Proc Natl Acad Sci U S A.* 2011; 108:5765–5770. [PubMed: 21436051]
22. Starr TK, Largaespada DA. Cancer gene discovery using the Sleeping Beauty transposon. *Cell Cycle.* 2005; 4:1744–1748. [PubMed: 16294016]
23. Perna D, Karreth FA, Rust AG, Perez-Mancera PA, Rashid M, Iorio F, Alifrangis C, Arends MJ, Bosenberg MW, Bollag G, et al. BRAF inhibitor resistance mediated by the AKT pathway in an Sleeping Beauty transposon insertional mutagenesis Moriarity and Largaespada 71 oncogenic BRAF mouse melanoma model. *Proc Natl Acad Sci U S A.* 2015; 112:E536–E545. [PubMed: 25624498]
24. Yachida S, Jones S, Bozic I, Antal T, Leary R, Fu B, Kamiyama M, Hruban RH, Eshleman JR, Nowak MA, et al. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature.* 2010; 467:1114–1117. [PubMed: 20981102]
25. Janik CL, Starr TK. Identification of Sleeping Beauty transposon insertions in solid tumors using linker-mediated PCR. *J Vis Exp.* 2013:e50156. [PubMed: 23407503]
26. Riordan JD, Drury LJ, Smith RP, Brett BT, Rogers LM, Scheetz TE, Dupuy AJ. Sequencing methods and datasets to improve functional interpretation of sleeping beauty mutagenesis screens. *BMC Genomics.* 2014; 15:1150. [PubMed: 25526783]
27. Mann KM, Ward JM, Yew CC, Kovochich A, Dawson DW, Black MA, Brett BT, Scheetz TE, Dupuy AJ, Chang DK, et al. Sleeping Beauty mutagenesis reveals cooperating mutations and pathways in pancreatic adenocarcinoma. *Proc Natl Acad Sci U S A.* 2012; 109:5934–5941. [PubMed: 22421440]
28. Berquam-Vrieze KE, Nannapaneni K, Brett BT, Holmfeldt L, Ma J, Zagorodna O, Jenkins NA, Copeland NG, Meyerholz DK, Knudson CM, et al. Cell of origin strongly influences genetic selection in a mouse model of T-ALL. *Blood.* 2011; 118:4646–4656. [PubMed: 21828136]
29. Collier LS, Adams DJ, Hackett CS, Bendzick LE, Akagi K, Davies MN, Diers MD, Rodriguez FJ, Bender AM, Tieu C, et al. Whole-body sleeping beauty mutagenesis can cause penetrant leukemia/lymphoma and rare high-grade glioma without associated embryonic lethality. *Cancer Res.* 2009; 69:8429–8437. [PubMed: 19843846]
30. March HN, Rust AG, Wright NA, ten Hoeve J, de Ridder J, Eldridge M, van der Weyden L, Berns A, Gadiot J, Uren A, et al. Insertional mutagenesis identifies multiple networks of cooperating genes driving intestinal tumorigenesis. *Nat Genet.* 2011; 43:1202–1209. [PubMed: 22057237]
31. van der Weyden L, Giotopoulos G, Rust AG, Matheson LS, van Delft FW, Kong J, Corcoran AE, Greaves MF, Mullighan CG, Huntly BJ, et al. Modeling the evolution of ETV6-RUNX1-induced B-cell precursor acute lymphoblastic leukemia in mice. *Blood.* 2011; 118:1041–1051. [PubMed: 21628403]
32. Keng VW, Sia D, Sarver AL, Tschida BR, Fan D, Alsinet C, Sole M, Lee WL, Kuka TP, Moriarity BS, et al. Sex bias occurrence of hepatocellular carcinoma in Poly7 molecular subclass is associated with EGFR. *Hepatology.* 2013; 57:120–130. [PubMed: 22899566]
33. Koso H, Takeda H, Yew CC, Ward JM, Nariai N, Ueno K, Nagasaki M, Watanabe S, Rust AG, Adams DJ, et al. Transposon mutagenesis identifies genes that transform neural stem cells into glioma-initiating cells. *Proc Natl Acad Sci U S A.* 2012; 109:E2998–E3007. [PubMed: 23045694]
34. O'Donnell KA, Keng VW, York B, Reineke EL, Seo D, Fan D, Silverstein KA, Schrum CT, Xie WR, Mularoni L, et al. A Sleeping Beauty mutagenesis screen reveals a tumor suppressor role for Ncoa2/Src-2 in liver cancer. *Proc Natl Acad Sci U S A.* 2012; 109:E1377–E1386. [PubMed: 22556267]
35. van der Weyden L, Arends MJ, Rust AG, Poulgiannis G, McIntyre RE, Adams DJ. Increased tumorigenesis associated with loss of the tumor suppressor gene Cadml. *Mol Cancer.* 2012; 11:29. [PubMed: 22553910]

36. Genovesi LA, Ng CG, Davis MJ, Remke M, Taylor MD, Adams DJ, Rust AG, Ward JM, Ban KH, Jenkins NA, et al. Sleeping Beauty mutagenesis in a mouse medulloblastoma model defines networks that discriminate between human molecular subgroups. *Proc Natl Acad Sci U S A*. 2013; 110:E4325–E4334. [PubMed: 24167280]
37. Lastowska M, Al-Afghani H, Al-Balool HH, Sheth H, Mercer E, Coxhead JM, Redfern CP, Peters H, Burt AD, Santibanez-Koref M, et al. Identification of a neuronal transcription factor network involved in medulloblastoma development. *Acta Neuropathol Commun*. 2013; 1:35. [PubMed: 24252690]
38. Quintana RM, Dupuy AJ, Bravo A, Casanova ML, Alameda JP, Page A, Sanchez-Viera M, Ramirez A, Navarro M. A transposon-based analysis of gene mutations related to skin cancer development. *J Invest Dermatol*. 2013; 133:239–248. [PubMed: 22832494]
39. Tang JZ, Carmichael CL, Shi W, Metcalf D, Ng AP, Hyland CD, Jenkins NA, Copeland NG, Howell VM, Zhao ZJ, et al. Transposon mutagenesis reveals cooperation of ETS family transcription factors with signaling pathways in erythro-megakaryocytic leukemia. *Proc Natl Acad Sci U S A*. 2013; 110:6091–6096. [PubMed: 23533276]
40. van der Weyden L, Rust AG, McIntyre RE, Robles-Espinoza CD, del Castillo Velasco-Herrera M, Strogantsev R, Ferguson-Smith AC, McCarthy S, Keane TM, Arends MJ, et al. Jdp2 downregulates Trp53 transcription to promote leukaemogenesis in the context of Trp53 heterozygosity. *Oncogene*. 2013; 32:397–402. [PubMed: 22370638]
41. Zanesi N, Balatti V, Riordan J, Burch A, Rizzotto L, Palamarchuk A, Cascione L, Lagana A, Dupuy AJ, Croce CM, et al. A Sleeping Beauty screen reveals NF- κ B activation in CLL mouse model. *Blood*. 2013; 121:4355–4358. [PubMed: 23591791]
42. Rogers LM, Olivier AK, Meyerholz DK, Dupuy AJ. Adaptive immunity does not strongly suppress spontaneous tumors in a Sleeping Beauty model of cancer. *J Immunol*. 2013; 190:4393–4399. [PubMed: 23475219]
43. Bard-Chapeau EA, Nguyen AT, Rust AG, Sayadi A, Lee P, Chua BQ, New LS, de Jong J, Ward JM, Chin CK, et al. Transposon mutagenesis identifies genes driving hepatocellular carcinoma in a chronic hepatitis B mouse model. *Nat Genet*. 2014; 46:24–32. [PubMed: 24316982]
44. Been RA, Linden MA, Hager CJ, DeCoursin KJ, Abrahante JE, Landman SR, Steinbach M, Sarver AL, Largaespada DA, Starr TK. Genetic signature of histiocytic sarcoma revealed by a sleeping beauty transposon genetic screen in mice. *PLOS ONE*. 2014; 9:e97280. [PubMed: 24827933]
45. Vyazunova I, Maklakova VI, Berman S, De I, Steffen MD, Hong W, Lincoln H, Morrissy AS, Taylor MD, Akagi K, et al. Sleeping Beauty mouse models identify candidate genes involved in gliomagenesis. *PLOS ONE*. 2014; 9:e113489. [PubMed: 25423036]
46. Koso H, Tshako A, Lyons E, Ward JM, Rust AG, Adams DJ, Jenkins NA, Copeland NG, Watanabe S. Identification of FoxR2 as an oncogene in medulloblastoma. *Cancer Res*. 2014; 74:2351–2361. [PubMed: 24599127]
47. Mann MB, Black MA, Jones DJ, Ward JM, Yew CC, Newberg JY, Dupuy AJ, Rust AG, Bosenberg MW, McMahon M, et al. Transposon mutagenesis identifies genetic drivers of Braf melanoma. *Nat Genet*. 2015

Figure 1.

Basic structure of a *SB* cancer screen. Most screens to date utilize mice carrying the conditional SB11 *Rosa26* “knockin” allele (*Rosa26-LSL-SB11*) and T2/Onc concatemer, which are crossed to mice carrying a tissue specific Cre transgene (TSP-Cre) and in many cases some cancer predisposing mutation (e.g. *LSL-Trp53^{R270H}*). Important choices to be made at each step of such a screen are boxed.

Figure 2.

Potential future refinements to *SB* cancer screens. Improvements and refinements to *in vivo* cancer screening using *SB* might be made at the level of the mouse, analysis of the primary tumor, or single cells isolated from the primary tumor.

Table 1

Sleeping Beauty (SB) based screens for cancer genes *in vivo* published to date. Beginning with the oldest papers, the first author, year of publication and title are shown as well as primary tissue/cancer type targeted in the work. References [9^{••},10^{••},11^{••},13^{••},14,16[•],17,18,19[•],20,21,23,27–47]

Author/year	Year	Cancer Type	Title
Dupuy <i>et al.</i> ,	2005	Blood	Mammalian mutagenesis using a highly mobile somatic Sleeping Beauty transposon system.
Collier <i>et al.</i> ,	2009	Blood	Whole-body sleeping beauty mutagenesis can cause penetrant leukemia/lymphoma and rare high-grade glioma without associated embryonic lethality.
Keng <i>et al.</i> ,	2009	Liver	A conditional transposon-based insertional mutagenesis screen for genes associated with mouse hepatocellular carcinoma.
Starr <i>et al.</i> ,	2009	Colorectal	A transposon-based genetic screen in mice identifies genes altered in colorectal cancer.
Berquam-Vrieze <i>et al.</i> ,	2011	Blood	Cell of origin strongly influences genetic selection in a mouse model of T-ALL.
Collier <i>et al.</i> ,	2011	Sarcoma	Cancer gene discovery in solid tumors using transposon-based somatic mutagenesis in the mouse.
Karreth <i>et al.</i> ,	2011	Skin	<i>In vivo</i> identification of tumor-suppressive PTEN ceRNAs in an oncogenic BRAF-induced mouse model of melanoma.
Koudijs <i>et al.</i> ,	2011	Blood	High-throughput semiquantitative analysis of insertional mutations in heterogeneous tumors.
March <i>et al.</i> ,	2011	Colorectal	Insertional mutagenesis identifies multiple networks of cooperating genes driving intestinal tumorigenesis.
Starr <i>et al.</i> ,	2011	Colorectal	A Sleeping Beauty transposon-mediated screen identifies murine susceptibility genes for adenomatous polyposis coli (Apc)-dependent intestinal tumorigenesis.
van der Weyden <i>et al.</i> ,	2011	Blood	Modeling the evolution of ETV6-RUNX1-induced B-cell precursor acute lymphoblastic leukemia in mice.
Keng <i>et al.</i> ,	2012	Liver	Sex bias occurrence of hepatocellular carcinoma in Poly7 molecular subclass is associated with EGFR.
Koso <i>et al.</i> ,	2012	Nervous system	Transposon mutagenesis identifies genes that transform neural stem cells into glioma-initiating cells.
Mann <i>et al.</i> ,	2012	Pancreas	Sleeping Beauty mutagenesis reveals cooperating mutations and pathways in pancreatic adenocarcinoma.
O'Donnell <i>et al.</i> ,	2012	Liver	A Sleeping Beauty mutagenesis screen reveals a tumor suppressor role for Ncoa2/ Src-2 in liver cancer.
Pérez-Mancera <i>et al.</i> ,	2012	Pancreas	The deubiquitinase USP9X suppresses pancreatic ductal adenocarcinoma.
van der Weyden <i>et al.</i> ,	2012	Blood	Increased tumorigenesis associated with loss of the tumor suppressor gene Cadm1.
Wu <i>et al.</i> ,	2012	Nervous system	Clonal selection drives genetic divergence of metastatic medulloblastoma.
Genovesi <i>et al.</i> ,	2013	Nervous system	Sleeping Beauty mutagenesis in a mouse medulloblastoma model defines networks that discriminate between human molecular subgroups.
Lastowska <i>et al.</i> ,	2013	Nervous system	Identification of a neuronal transcription factor network involved in medulloblastoma development.
Quintana <i>et al.</i> ,	2013	Skin	A transposon-based analysis of gene mutations related to skin cancer development.
Rahrmann <i>et al.</i> ,	2013	Nervous system	Forward genetic screen for malignant peripheral nerve sheath tumor formation identifies new genes and pathways driving tumorigenesis.
Tang <i>et al.</i> ,	2013	Blood	Transposon mutagenesis reveals cooperation of ETS family transcription factors with signaling pathways in erythro-megakaryocytic leukemia.
van der Weyden <i>et al.</i> ,	2013	Blood	Jdp2 downregulates Trp53 transcription to promote leukaemogenesis in the context of Trp53 heterozygosity.
Zanesi <i>et al.</i> ,	2013	Blood	A Sleeping Beauty screen reveals NF-kB activation in CLL mouse model.

Author/year	Year	Cancer Type	Title
Rogers <i>et al.</i> ,	2013	Mixed	Adaptive immunity does not strongly suppress spontaneous tumors in a Sleeping Beauty model of cancer.
Bard-Chapeau <i>et al.</i> ,	2014	Liver	Transposon mutagenesis identifies genes driving hepatocellular carcinoma in a chronic hepatitis B mouse model.
Been <i>et al.</i> ,	2014	Sarcoma	Genetic signature of histiocytic sarcoma revealed by a sleeping beauty transposon genetic screen in mice.
Vyazunova <i>et al.</i> ,	2014	Nervous system	Sleeping Beauty Mouse Models Identify Candidate Genes Involved in Gliomagenesis
Koso <i>et al.</i> ,	2014	Nervous system	Identification of FoxR2 as an oncogene in medulloblastoma.
Takeda <i>et al.</i> ,	2015	Colorectal	Transposon mutagenesis identifies genes and evolutionary forces driving gastrointestinal tract tumor progression.
Perna <i>et al.</i> ,	2015	Skin	BRAF inhibitor resistance mediated by the AKT pathway in an oncogenic BRAF mouse melanoma model.
Mann <i>et al.</i> ,	2015	Skin	Transposon mutagenesis identifies genetic drivers of <i>Braf</i> V600E melanoma.
Moriarity <i>et al.</i> ,	2015	Bone	A <i>Sleeping Beauty</i> Forward Genetic Screen Identifies New Genes and Pathways driving Osteosarcoma Development and Metastasis.

Sleeping Beauty mouse Models Identify Candidate Genes Involved in Gliomagenesis, PLoS One 2014 (Pubmed ID: 25423036).