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Advances in functional genetic screening with transposons and CRISPR/Cas9 to illuminate cancer biology

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Abstract

Large-scale genome sequencing studies have identified a wealth of mutations in human tumors and have dramatically advanced the field of cancer genetics. However, the functional consequences of an altered gene in tumor progression cannot always be inferred from mutation status alone. This underscores the critical need for complementary methods to assign functional significance to mutated genes in cancer. Transposons are mobile genetic elements that serve as powerful tools for insertional mutagenesis. Over the last decade, investigators have employed mouse models with ondemand transposon-mediated mutagenesis to perform unbiased genetic screens to identify clinically relevant genes that participate in the pathogenesis of human cancer. Two distinct DNA transposon mutagenesis systems, *Sleeping Beauty (SB)* and *PiggyBac (PB),* have been applied extensively *in vivo* and more recently, in *ex vivo* settings. These studies have informed our understanding of the genes and pathways that drive cancer initiation, progression, and metastasis. This review highlights the latest progress on cancer gene identification for specific cancer subtypes, as well as new technological advances and incorporation of the CRISPR/Cas9 toolbox into transposon-mediated functional genetic studies.

Introduction

Insertional mutagenesis screens in worms, flies, and mice have yielded fundamental discoveries in biology and led to the identification of critical signaling pathway components [1,2]. The molecular reconstruction of a *Tcl/mariner* DNA transposon from fish revolutionized the field and generated new opportunities for *in vivo* genome engineering. This re-awakened element, called *Sleeping Beauty* (*SB*), was the first synthetic transposon to be mobilized in mammalian cells [3●●,4]. Successful applications include germline and

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somatic mutagenesis in mice [5–9]. *PiggyBac* (*PB*), another DNA transposon originating from the cabbage looper moth, was subsequently shown to be active in mammalian cells and developed for *in vivo* somatic mutagenesis [10,11].

The *SB* and *PB* systems utilize two components, an engineered transposon that harbors a mutagenic gene trap element, and an enzyme termed the transposase. When both components are present in the same cell, the transposase binds the inverted repeat/direct repeat sequences and catalyzes mobilization of the transposon. For *in vivo* studies, independent transgenic mouse lines are bred to induce transposon mobilization. Insertion of the transposon harboring the gene trap into a new genomic location disrupts gene function by introducing gain-of-function or loss-of-function mutations depending on the orientation and location of the element (Figure 1). Bioinformatic analysis identifies common insertion sites (CISs) representing genomic windows with more transposon insertions than predicted by chance [12–15], revealing genes that accelerate tumorigenesis when their functions are altered.

Remarkably, tumors that arise from transposon mutagenesis in mice accurately model the anatomical and histological features of human cancers. Early whole-body mutagenesis screens using a ubiquitous *SB* transposase knocked into the *Rosa26* locus identified genes that promote hematopoietic cancers and sarcomas [5,7]. This was followed by screens in the liver and gastrointestinal system using tissue-specific Cre mouse lines that direct expression of the *SB* transposase to individual tissues [16,17]. In recent years, screens have been performed for many other tumor types including melanoma, neurofibroma, medulloblastoma, breast, prostate, and thyroid cancers (Table 1). Transposon mobilization has been shown to promote tumorigenesis alone or in cooperation with sensitized genetic backgrounds.

Several important factors must be considered for any transposon screen, including the choice of the transposon gene trap and transposase, the selection of the Cre driver line used to induce tissue-specific expression of the transposase, the scale and duration of the genetic screen, and the specific methods used for the identification and analysis of transposon insertions (Figure 2). A number of excellent studies have examined important statistical considerations for transposon mutagenesis screens and will not be discussed in detail here [12,18,19].

Breast cancer

Significant effort has focused on the identification of genes that contribute to breast cancer pathogenesis. Several key advantages of insertional mutagenesis over other functional approaches can be highlighted using recent *Sleeping Beauty* studies as examples. Invasive lobular carcinoma (ILC), the second most common breast cancer subtype, is characterized by loss of E-cadherin (*CDH1*). *Cdh1* null mice do not develop mammary tumors, suggesting that additional mutations are required for ILC development. One *SB* screen performed in mammary-specific *Cdh1* deficient mice uncovered recurrent and mutually exclusive insertions in genes implicated in actin cytoskeleton regulation in ILC [20••]. This revealed driver mutations that were not readily identified in human datasets. For example,

heterozygous inactivating insertions were identified in *Nonmuscle myosin Ha heavy chain 9* (*Myh9*), resulting in dosage reduction of MYH9. Yet, driver mutations in *MYH9* were not readily detected in human ILC because the gene is rarely mutated or exhibits shallow deletions, which are putative heterozygous deletions. Analysis of human ILCs revealed that *MYH9* was commonly altered by heterozygous copy number loss, which correlated with reduced expression of *MYH9* mRNA. Experimental validation confirmed that *Myh9* haploinsufficiency induced ILC formation *in vivo.* Consistent with this, it has been proposed that haploinsufficiency of candidate genes in commonly deleted regions may have detectable tumor suppressing activity only in the context of cooperating genetic events [21,22]. Given the heterogeneity of transposon-induced tumors which mimics the complexity of human cancers, similar screens may uncover additional context-dependent alterations in other tumor types.

Another advantage provided by a recent *SB* transposon mutagenesis study was the ability to pinpoint two candidate cancer genes (*Transformation related protein 53 binding protein 2* (*Trp53bp2*) and *Protein Phosphatase 1 Regulatory Subunit 12B* (*Ppp1r12b*), encoding Protein Phosphatase PP1-targeting subunits) with orthologues that are present in a large region on human chromosome 1q, which is known to be frequently amplified in human breast cancer [20••]. The large size of this amplicon made it particularly difficult to identify critical driver gene(s). Subsequent validation studies showed that *SB* insertions in these two genes caused truncation of the PP1-targeting subunits, and that expression of the truncated subunits induced tumor formation in genetically engineered mice. This demonstrates the power of integrating CIS gene lists with available copy number data to prioritize driver genes that are present in large windows of amplifications or deletions in human cancer.

SB screens on different mutant backgrounds identified additional breast cancer susceptibility genes. Classification of *SB*-induced tumors in *Pten* mutant mice identified a collection of different breast cancer subtypes, including basal-like (triple negative), luminal A, and HER2 positive tumors [23]. Functional validation studies identified eight tumor suppressor genes, including *Transcriptional Repressor GATA Binding* 1 (*Trps 1*) as a metastasis tumor suppressor in triple negative breast cancer (TNBC). Interestingly, multiple independent breast cancer (BC) screens used the K5-Cre transgenic mouse line to drive expression of the *SB* transposase [23–25]. The K5 Cre drives expression in all mammary epithelium cell populations, including basal cells and luminal cells, that likely contributed to the development of different breast cancer subtypes in these studies.

Chen and colleagues performed *SB* mutagenesis in breast epithelial cells alone or in combination with stabilized N-terminally truncated β-catenin [24]. Integration of this screening approach with survival prediction analysis led to the identification of six gene pairs with prognostic value that could stratify breast cancer subtypes. This demonstrates the utility of incorporating functional mutagenesis screens with expression and survival data to identify novel subtyping biomarkers.

Liver cancer

One of the most frequently modeled tumors using the *SB* system is hepatocellular carcinoma (HCC). This is likely due to the availability of excellent mouse models, prevalence of the disease, and accessibility of tumor tissues for genomic and histopathologic analysis. The first liver-specific screen for HCC, performed on a mutant *Tp53* background, identified known driver genes (*Epidermal growth factor receptor, Egfr,* and *Tyrosine-protein kinase Met*) and new potential therapeutic targets including *Ubiquitin conjugating enzyme E2H* (*Ube2h*) [16]. *Retrotransposon-like 1 (Rtl1),* initially identified in a T2/Onc3-induced screen [26], was subsequently validated as a novel gene that promotes HCC development [27]. Another study identified genes that cooperate with *MYC* to accelerate liver tumorigenesis, revealing a tumor suppressor role for Steroid Receptor Coactivator 2/Nuclear Receptor Coactivator 2 (Src-2/Ncoa2) [28]. Interestingly, SRC-2 promotes survival and metastasis in other tumor types, suggesting a tissue-specific and context-dependent role for SRC-2 in tumorigenesis [29]. Suresh and colleagues recently illuminated the mechanisms of tumor suppression by SRC-2 in liver and provided evidence that SRC-2 may exhibit oncogenic or tumor suppressor activity depending on the target genes and nuclear receptors that are expressed in distinct tissues [30].

Chronic infection with hepatitis B virus (HBV) is the most common risk factor for developing HCC. To identify genes that cooperate with HBV-induced liver inflammation in HCC development, Jenkins and colleagues performed a *SB* mutagenesis screen using transgenic mice expressing the HBV surface antigen (HBsAg) [31]. This near-saturating screen identified early-stage and late-stage drivers of tumorigenesis, including many CIS genes involved in cellular metabolic processes. Recently, two studies have been performed in the context of chronic liver damage and hepatic steatosis, recapitulating additional settings in which HCC frequently develops [32,33]. Interestingly, chronic liver injury enhanced tumor penetrance and significantly altered *SB* insertion profiles, reflecting distinct selective pressures exhibited by this tumor type.

PiggyBac mutagenesis

PiggyBac serves as a complementary mutagenesis system to *SB* and has several important distinctions. First, *SB* integrates into 'TA' dinucleotides whereas *PB* requires 'TTAA' motifs that occur less frequently in mammalian genomes. Second, *SB* has no preference for insertion into genes [34], while *PB* more frequently integrates into active transcription units [35–37]. Third, *PB* transposase activity is more efficient in mammalian genomes and has the capability to mobilize larger payloads. Fourth, unlike *SB, PB* mobilizes without creating footprint mutations. This means that an excision event restores the DNA sequence that existed before the insertion occurred. While this may cause less genomic damage during mutagenesis, it may also preclude the identification of some *PB* insertions. Finally, *SB* exhibits more local hopping, whereby the transposon favors mobilization to sites in proximity to the donor concatemer in transgenic mice. This allows for a higher mutational coverage across the genome and may be exploited for targeted regional mutagenesis. However, Rad and colleagues have suggested that *PB* may be superior for regional

mutagenesis because it produces fewer nonspecific insertions around the donor locus [38••].

Although fewer *PB* screens have been performed to date, a versatile *PB* mutagenesis platform was generated by developing a series of transgenic mouse lines that carry different transposon constructs. Most transposon lines carry *PB* and *SB* inverted terminal repeats, allowing mobilization with either transposase [11,38••]. Transgenic lines with three distinct promoter/enhancer elements were generated, each with the transposon concatemer present in a broad range of copies and on different chromosomes. Interestingly, the selection of the promoter used in the transposon line skews the tumor spectrum in whole body screens, with the murine stem cell virus (MSCV) 5'LTR inducing primarily hematopoietic cancers, the CMV early enhancer/chicken beta actin (CAG) promoter producing more solid tumors, and the phosphoglycerate kinase (PGK) promoter generating a mix of both tumor types. This phenomenon has also been observed in *SB* screens. Early *SB* screens using the *T2/Onc* or *T2/Onc2* mouse strains, which utilized the MSCV promoter, exhibited a tendency to develop hematopoietic tumors. Replacing the MSCV promoter with the CAG or PGK promoters increased the incidence of solid tumors [26]. This modularity provides investigators great flexibility by offering the ability to alter the tumor type and incidence when performing whole body or tissue-specific *SB* and *PB* screens.

A conditional *PB* mouse model identified novel oncogenic networks including FOXP1 as an oncogenic transcription factor in pancreatic cancer [39••]. This screen also helped elucidate the genetic basis of different histologic subtypes of pancreatic cancer. Insertions in *Fidgetin* (*Fign*), which encodes a member of the AAA-ATPase superfamily, were significantly enriched in hepatoid tumors, a rare pancreatic cancer subtype. These results implicate altered regulation of *Fign* in the development of hepatoid pancreatic cancer, however direct evidence for this awaits validation. Interestingly, hepatoid cancers have not been described in pancreas-specific *SB* screens [40,41], and the identified CISs from these *SB* screens only partially overlapped with candidate cancer genes found in the conditional *PB* pancreatic cancer screen. This is likely due to different integration preferences, highlighting the complementarity of these two systems.

In addition to *SB* and *PB*, other transposable elements have been mobilized in mouse cells including *Tol2, Minos,* and a codon-optimized mouse LINE-1 retrotransposon, *ORFeus* [6,42]. Application of these systems in future studies may further expand the repertoire of available insertional mutagenesis platforms for cancer gene discovery.

In contrast to transposon mutagenesis, chemical mutagenesis is another complementary method with unique features, most notably the capability to induce point mutations that are typically not modeled by insertional mutagenesis approaches [43]. Although next-generation sequencing allows genome-wide detection of chemically-induced mutations, existing limitations include prohibitive costs and high background mutation rates.

Technological advances and *ex vivo* screens

Since the advent of transposon mutagenesis, numerous technological advances in the recovery of insertions and identification of fusion transcripts have been made. Most transposon screens utilize ligation-mediated PCR (LM-PCR) to recover transposon insertions from tumor DNA. However, the use of restriction enzymes in the LM-PCR reaction generates bias due to uneven distribution of restriction sites across the genome. Shearing of genomic DNA before LM-PCR increases the insertion recovery and allows 'truncal' insertions that occur early in tumor development to be distinguished from 'branch' insertions that occur later in tumor development [44]. Recently, a semi-quantitative transposon insertion site sequencing method using acoustic DNA shearing (QiSeq) has been described [38••]. Further refinement of sequencing methods has uncovered transposon insertions in single tumor cells (SBCapSeq) [45•]. This powerful method detected clonal insertion events in a myeloid leukemia mouse model and identified cooperating events in individual tumor cells. RNA sequencing of *SB*-induced tumors has been particularly useful for identifying fusion transcripts between the transposon elements and endogenous transcripts in order to determine whether a specific *SB* insertion generates a gain-of-function or loss-of-function mutation [46].

Another clever screening approach combined targeted gene inactivation to single-copy transposon mobilization to identify novel genes that cooperate with *Pten* in suppressing prostate, breast, and skin tumorigenesis [47••]. There are several unique benefits to using this model. First, a single-copy *SB* transposon limited the number of insertions to one per cell, which may reduce the number of passenger insertions. Second, transposition occurred simultaneously in the same cells that underwent *Pten* inactivation, potentially enhancing the sensitivity of the screen. Finally, unlike most other *SB* screens, the gene trap promoted only inactivating mutations but not activating mutations. Although this precluded the identification of putative oncogenes, this greatly simplified the interpretation of the roles of uncharacterized CIS genes as putative tumor suppressors. Additional studies utilizing this approach are warranted to gain a better understanding of how altering transposon gene-trap choice and copy number impacts the presence of passenger mutations and degree of tumor heterogeneity.

SB and *PB* screens have been employed to identify genes that drive metastasis [39••,48,49] and therapy resistance [50–52]. Recently, Morrissy and colleagues developed a *SB*-driven mouse model of metastatic medulloblastoma that recapitulated post-treatment tumor recurrence. The investigators performed a mutagenesis screen in mice to identify genes that drive primary medulloblastoma, then resected tumors and employed image-guided radiotherapy, which is the standard therapy for children with this disease [53•]. Sequencing of *SB*-induced tumors revealed a poor degree of overlap between the *SB* insertions in primary mouse tumors and the insertions in tumors that recurred after treatment. Consistent with this, whole genome sequencing of human tumors at the time of diagnosis and at recurrence revealed significant genetic divergence. Striking results from the mouse and human data showed that the dominant clone at recurrence arose in part through clonal selection of a minor clone that was present at the time of diagnosis. This suggests that surgery and radiation generate evolutionary pressure, thereby allowing divergent clones to

become resistant to therapies. The authors proposed that therapeutic strategies targeting truncal mutations in the primary tumor are destined to fail if the mutation is lacking after recurrence. They also advocated that sequencing of biopsies at recurrence should guide future clinical trials and treatment decisions. In the future, transposon-based mutagenesis systems may be further refined to screen for better drug targets at recurrence for different tumor types.

Although *in vivo* transposon screens have proven to be effective for cancer gene discovery, they are also time and resource intensive. This prompted several groups to develop *ex vivo* based systems wherein human or mouse cells growing in culture are mutagenized and screened for the acquisition of specific phenotypes *in vitro* or *in vivo.* This approach relies upon stable or transient expression of the transposase and is easily modified for conditional or dose-dependent expression of the transposon or transposase in different settings. This also avoids local hopping, inherent to *SB* and *PB* screens. Mutagenesis of human bone explant mesenchymal cells utilizing a hybrid lentiviral and *SB* mutagenesis system generated myxofibrosarcomas when transplanted into mice [54]. Other cell-based screens have identified genes involved in transformation of neural stem cells into glioma-initiating cells [55] and genes driving epithelial-mesenchymal transition in immortalized mouse hepatoblasts [56]. One of the largest *ex vivo SB* screens performed to date identified genes that promote growth factor independence and transformation of Ba/F3 cells, an IL-3-dependent murine pro-B cell line. Recurrent insertions were identified in JAK/STAT and MAPK pathway genes in addition to a large number of genes that are mutated or associate with survival of leukemia patients but had not previously been linked to these pathways [57●]. Enforced expression of individual CIS genes promoted growth factor independence and tumori-genesis *in vivo*, validating this approach. Finally, an *ex vivo* recellularized human colon model identified genes that drive colorectal cancer progression [58●]. This advance contributed a unique tissue-engineering method and enabled the implementation of forward genetic screening in human tissues under physiologic conditions.

Incorporation of the CRISPR/Cas9 mutagenesis toolbox into transposon-mediated screens

The clustered regularly interspaced short palindromic repeats/Cas9 (CRISPR/Cas9) mutagenesis system has emerged as a revolutionary tool for interrogating phenotypes and pathways in unbiased high-throughput screens [59,60]. Several groups have incorporated the CRISPR toolbox into transposon studies by using *PB* and *SB* to deliver individual single guide RNAs (sgRNAs) to mice. For example, Weber and colleagues generated a *CRISPR-SB* vector with sgRNA and Cas9 cassettes flanked by *SB* inverted repeats, generating a system for multiplexed mutagenesis of large gene sets in adult mice [61]. When the *CRISPR-SB* and *SB* transposase vectors were delivered via tail vein injection, animals developed HCC and intrahepatic cholangiocarcinoma (ICC). *PB* was also identified as an efficient delivery vehicle for *in vivo* CRISPR library screening in mice [62].

One major difference between CRISPR screening and transposon screening is that Cas9 derivatives are engineered for the identification of gain-of-function (GOF) or loss-of-

function (LOF) mutations, but not at the same time. In contrast, transposon screening allows for simultaneous identification of GOF and LOF mutations in the same mutagenized tumor or clone, which may more accurately reflect the complexity of human tumors. Furthermore, transposon-mediated delivery provides a non-viral alternative for efficient delivery of CRISPR libraries in mice and for cell-based screens.

Concluding remarks

One understudied advantage provided by transposon mutagenesis screens over cDNA overexpression or RNA interference screens is that important non-coding and regulatory regions of the genome may be detected in an unbiased manner. To date, the vast majority of transposon screens have focused on validating protein-coding genes. Two notable exceptions include the identification of competing endogenous RNAs (ceRNAs) that suppress *Pten* in a $Braf^{V600E}$-induced mouse model of melanoma [63] and the identification of a *Cdkn2a cis*-regulatory region in a recent conditional *PB* pancreatic cancer screen [39••]. In future studies, transposon screens should be harnessed to identify and rigorously validate non-coding RNAs and critical regulatory regions that drive cancer progression. However, this will depend on the extent to which mouse and human non-coding RNAs exhibit significant conservation at the primary sequence level.

In summary, transposon mutagenesis studies have provided important insights into the functional consequences of mutated genes in human tumors. As new technologies continue to emerge, these studies will undoubtedly improve our understanding of clonal evolution in primary tumors and metastases, the genetic basis of histologic subtypes for different tumors, and mechanisms underlying drug resistance. However, several key questions remain. For example, can we continue to improve and refine the *SB* and *PB* systems? One current limitation of transposon mutagenesis is that it does not faithfully reproduce the full spectrum of mutations seen in human cancer, namely point mutations and reciprocal translocations. In the future, innovative modifications to these systems may be developed for this purpose. Also, what is the best way to integrate the CIS gene identification with human genomic data? One possibility is to link transposon insertion data and associated databases [64,65] with existing Cancer Genome Atlas datasets (TCGA) and oncogenomic databases including cBioPortal, the Catalog of Somatic Mutations in Cancer (COSMIC), Oncomine, and the International Cancer Genome Consortium (ICGC). Direct integration of available functional data with human clinical datasets will accelerate the development of novel diagnostic and therapeutic strategies for human malignancies.

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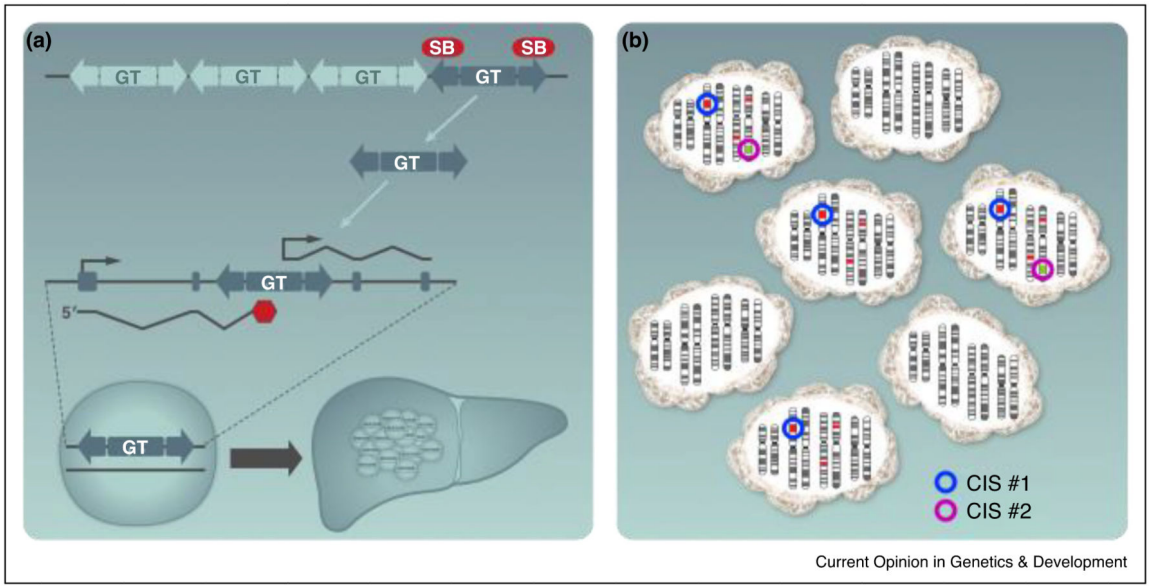

Figure 1. Transposon mobilization disrupts gene function by introducing gain-of-function or loss-of-function mutations.

(a) The *SB* and *PB* systems utilize two transgenic mouse lines, one harboring a concatemerized mutagenic gene trap (GT) that can disrupt gene function, and a second line carrying a ubiquitous or tissue-specific transposase that binds the transposon ends and catalyzes mobilization to new genomic sites. *SB* mutagenesis is depicted in liver cells, leading to the formation of liver tumors *in vivo.* The gene trap can alter gene function in two ways. In one or both orientations, a splice acceptor is followed by a polyadenylation (pA) signal. When the transposon inserts into a gene, the gene trap may splice to the transcript and the pA signal will prematurely truncate the mRNA, thereby disrupting expression of a candidate tumor suppressor. Additionally, a strong promoter followed by a splice donor (SD) is usually present in only one orientation. Transposon insertions that utilize this promoter/SD may drive overexpression of candidate oncogenes. **(b)** Bioinformatic analysis identifies common insertion sites (CISs) that represent genomic windows with more transposon insertions than predicted by chance. CISs 1 and 2 (represented as blue and purple circles) are found in independent tumors. Different methods of CIS identification are used to uncover genes that accelerate tumorigenesis, including Monte Carlo-based and Poisson distribution methods, Gaussian Kernel Convolution methods, and gene-centric common insertion site (gCIS) analysis.

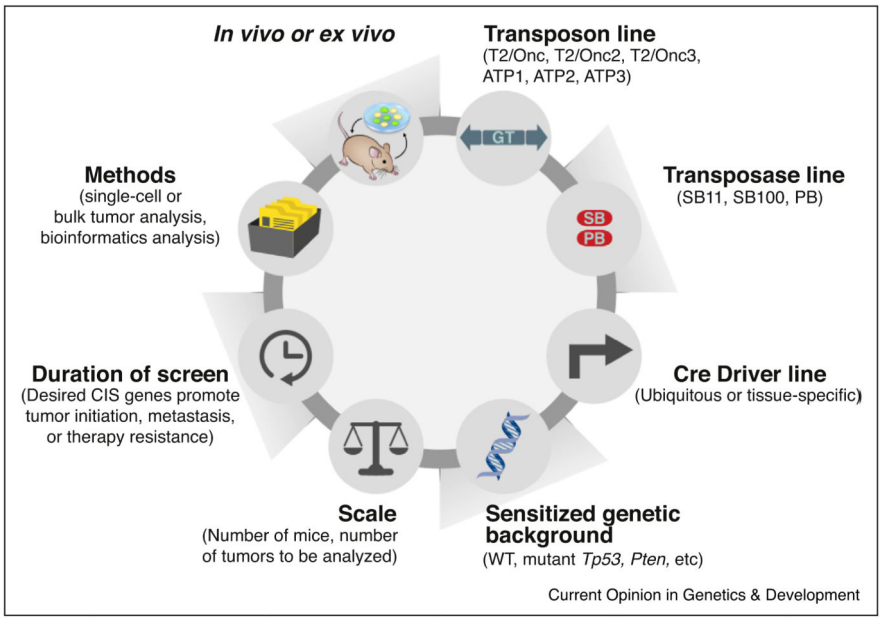

Figure 2. Important considerations for performing transposon screens.

Critical considerations for performing transposon screens include: 1) the selection of the transposon gene trap vector (*SB* lines = T2/Onc, T2/Onc2, T2/Onc3; *PB* lines = ATP1, ATP2, ATP3 that differ based on the transposon copy number within the concatemer and site of integration on different chromosomes), 2) the transposase line, 3) the selection of the Cre driver line used to induce expression of the transposase, 4) whether transposon mutagenesis occurs alone or in combination with a sensitized genetic background, 5) the scale of the screen (depending on the number of mice and tumors that are desired for analysis), 6) the duration of the screen (depending on what types of CIS genes are desired at early or late stages of tumorigenesis), 7) the specific methods used for the identification and analysis of transposon insertions, and 8) whether *in vivo* or *ex vivo* mutagenesis is desired.

Table 1

Transposon mutagenesis screens published after 2014

Tissue/Tumor type	Transposon system	Sensitized background/System	Reference
Invasive lobular breast carcinoma	*Rosa26Lox66SBLox71;T2/Onc*	*Cdh1* mutant	[20]
Triple negative breast cancer	*Rosa26-LSL* SBase; T2/Onc2 and T2/Onc3	*Pten* mutant	[23]
Triple negative breast cancer	*Rosa26*-LSL SBase; T2/Onc2	Truncated beta-catenin	[24]
Breast cancer	*Rosa26*-LSL SBase; T2/Onc2	$Trp53^{+/-}$	[25]
Chronic liver injury	*Rosa26*-LSL SBase; T2/Onc3	CCl4 treatment	[32]
Steatosis-associate hepatic tumors	*Rosa26*-LSL SBase; T2/Onc	High fat diet	[33]
Pancreatic cancer	$Rosa26^{LSL\text{-}PB}$; *ATP1-S2*	$Kras^{LSL\text{-}G12D}$	[39]
Metastatic prostate cancer	$Rosa26^{Lox66SBLox71}$; *T2/Onc3*	*Pten* mutant	[48]
Osteosarcoma	*Rosa26-LSL* SBase; T2/Onc	LSL-$Trp53^{R270H}$	[49]
Medulloblastoma recurrence	*Math1-SB11;* T2/Onc and T2/Onc2	$Ptch^{+/-}$	[53]
Lung cancer	*Rosa26*-LSL SBase; T2/Onc and T2/Onc2	LSL-$Trp53^{R270H}$; $p19^{ARF-/-}$; *Pten* mutant	[66]
B-cell acute lymphoblastic leukemia	*Rosa26*-LSL SBase; T2/Onc	*Stat5b-CA*	[67]
Melanoma	Rosa26-LSL SBase; T2/Onc2 and T2/Onc3	$Braf^{V600E}$	[68]
Thyroid cancer	*Rosa26*-LSL SBase; T2/Onc2	$Hras^{G12V}$	[69]
Multiple intestinal neoplasia	*Rosa26*-LSL SBase; T2/Onc2	$Tgfbr2^{fl/fl}$	[70]
Gastric cancer	*Rosa26*-LSL SBase; T2/Onc3	$Smad4^{KO}$/+	[71]
Gastrointestinal tract	$Rosa26^{Lox66SBLox71}$; *T2/Onc2*	Apc^{min}; $Kras^{G12D}$; $Smad4^{KO}$; $Tp53^{R172H}$	[72]
Neurofibroma	*Rosa26*-LSL SBase; T2/Onc	$Nf1^{fl/fl}$	[73]
New technologies			
Quantitative insertion site sequencing (QiSeq)	$Rosa26^{PB}$; $Rosa26^{LSL\text{-}PB}$; *ATP1–3*	$_{Kras}LSL\text{-}G12D$	[38]
Single-cell transposon insertion sequencing (SBCapSeq)	*Rosa26*-LSL SBase; T2/Onc2	$Tp53^{+/-}$; $Tp53^{LSL-R172H/+}$	[45]
RNA sequencing of SB-induced tumors	*Rosa26*-LSL SBase; T2/Onc	LSL-$Trp53^{R270H}$	[46]
Single-copy SB mutagenesis	*Rosa26*-LSL SBase; Inactivating transposon (ITP2m)	$Pten^{SBm2/+}$; $Blm^{m3/m3}$	[47]
Ex vivo screens			
Epithelial-mesenchymal transition in HCC	*Rosa26*-LSL SBase; T2/Onc2	*Pten* mutant	[56]
Growth factor independence and B-cell leukemogenesis	SB11; SB100; T2/Onc	Murine Ba/F3 cells	[57]
Colorectal cancer (recellularized human colon model)	SB100; modified T2/Onc	Human colonic epithelial cells (hCEC-APCshRNA)	[58]

Curr Opin Genet Dev. Author manuscript; available in PMC 2018 December 31.

Tissue/Tumor type	Transposon system	Sensitized background/System	Reference
Drug resistance			
Fludarabine resistance in chronic lymphocytic leukemia (CLL)	PB transposon; HyPBase transposase	Human CLL cells	[50]
Braf inhibitor resistance in melanoma	*Rosa26*-LSL SBase; T2/Onc	$Braf^{V618E}$	[51]
Transposon screens incorporating CRISPR/Cas9			
CRISPR/Cas9 somatic mutliplex mutagenesis	CRISPR-SB (sgRNA and Cas9); hSB5	$Kras^{LSL\text{-}G12D}$	[61]
PiggyBac *in vivo* CRISPR library screening	PB-CRISPRM1; PB-CRISPR-M2	CD-1	[62]

Additional references listed in Table 1 include [66–73]. *SB* mutagenesis screens published before 2015 have been reviewed previously [19,74].