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Sleeping Beauty transposon system for genetic etiological research and gene therapy of cancers

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Abbreviations: *Alb*-Cre, *Albumin* promoter-Cre; CAG promoter, CMV enhancer/chicken β-actin promoter; CAR, chimeric antigen receptor; CIS, common insertion site; CMV, chimeric cytomegalovirus; Cre, cyclization recombination enzyme; CRC, colorectal cancer; DDE, Asp, Asp, Glu; DMBA/TPA; 7, 12-dimethylbenzanthracene/12-O-tetradecanoylphorbol-13-acetate; DR, direct orientation; *Fah*, fumarylacetoacetate hydrolase gene; GWAS, gnome wide analysis study; HBV, Hepatitis B Virus; HBx, HBV X protein; HCC, hepatocellular carcinoma; IRs, inverted repeat sequences; LsL, *lox*P-stop-*lox*P; PAI, Pro, Ala, Ile; MPNSTs, malignant peripheral nerve sheath tumor; MSCV, murine stem cell virus; PBMCs, peripheral blood mononuclear cells; RED, Arg, Glu, Asp; *Rtl1*, Retrotransposon-like 1; *Rosa*SBaseLsL, Cre-inducible SBase allele; *SB*, *Sleeping Beauty*; SBase, *Sleeping Beauty* transposase; sgRNA, single guide RNA; *shp53*, short hairpin RNA against the *Trp53* gene; *StatinAE*, angiostatin-endostatin fusion gene; Trp53, transformation related protein 53.

Carcinogenesis is etiologically associated with somatic mutations of critical genes. Recently, a number of somatic mutations and key molecules have been found to be involved in functional networks affecting cancer progression. Suitable animal models are required to validate cancer-promoting or -inhibiting capacities of these mutants and molecules. *Sleeping Beauty* transposon system consists of a transposon that carries gene(s) of interest and a transposase that recognizes, excises, and reinserts genes in given location of the genome. It can create both gain-of-function and loss-of-function mutations, thus being frequently chosen to investigate the etiological mechanisms and gene therapy for cancers in animal models. In this review, we summarized current advances of *Sleeping Beauty* transposon system in revealing molecular mechanism of cancers and improving gene therapy. Understanding molecular mechanisms by which driver mutations contribute to carcinogenesis and metastasis may pave the way for the development of innovative prophylactic and therapeutic strategies against malignant diseases.

Introduction

Cancer is the leading cause of death in developed countries and the second leading cause of mortality in developing world.[1] Carcinogenesis is a long-term process as the human body is continuously exposed to physical, chemical, and biological carcinogenic factors and their complex interactions with genetic variations. It is, at least partly, attributable to the mutations in critical genes responsible for normal programming of cell proliferation, differentiation, and death. These cancer-inducing somatic mutations can be generally classified as driver and passenger mutations. Driver mutations are indispensible for cancer development. They provide pro-cancerous milieu and are positively selected in cancer evolution. It has been summarized that driver mutations in more than 120 genes contribute to the development of cancers.[2] However, most somatic mutations are passenger mutations. Passenger mutations accumulated in somatic cells through DNA replication are not subject to positive selection and not directly associated with carcinogenesis. Therefore, it is important to distinguish functional driver mutations from random passenger mutations during systematic mutation screening.[2] With the use of genome-wide association study (GWAS) and next-generation sequencing technologies, it is possible to study the complicated associations of genetic mutations with cancer occurrence and progression. Recently, the National Cancer Institute of USA have released the largest-ever database of cancer-related genetic variations, providing a comprehensive resource to investigate targeted treatments for cancers. This gradually enables personalized prophylaxis and treatment of malignant diseases. One challenge of novel molecular therapy is to perform replicable experiments in appropriate animal models before clinical trials. Nowadays, transposon systems are often applied to construct animal models. To choose a proper transposon system for the construction of animal models in cancer etiological research and cancer gene therapy is one of the key steps toward personalized medicine.

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Sleeping Beauty Transposon System

Transposon system

Transposon system is a non-viral DNA-mediated gene transfer system. It includes a transposase that is capable of recognizing, excising, and reinserting particular DNA sequences in targeted

locations of the genome. In the past decades, a number of transposable elements, including *Tc1*, *Tol2*, *Minos*, *Himar1*, *Hsmar1*, *Mos1*, *Frog Prince,* and *Piggyback,* in vertebrate cells have been developed.[3-9] Of those, *Sleeping Beauty* (*SB*) has been extensively characterized.

SB transposon system and its function

SB transposon system was created from Salmonid, which was first reported in 1997.[4] It was named the "*Sleeping Beauty*" because the transposase isolated from Salmonid fish was transpositionally inactive due to the accumulation of mutations and artificially reawaken by eliminating the inactivating mutations.[4,10-12] *SB* transposon system is a *Tc1*/*mariner*-type delivery system that consists of 2 components: the transposase (SBase) and the integration cassette (transposon). *SB* transposon has 210-250 bp inverted repeats (IRs) at their termini and directly repeated DNA sequence motifs (DRs) at the ends of each IR (termed as IR/DR domain). *SB* transposon can sandwich a desired genetic cargo within the IR/DR domains (**Fig. 1A**). SBase has several conserved domains that are critical for its function. At the N-terminus of SBase, a bipartite DNA-binding domain [PAI (Pro, Ala, Ile) and RED (Arg, Glu, Asp)] can confer specific binding to IRs because it overlaps with a nuclear localization signal (NLS) sequence. A domain directing cleavage and insertion as well as targeting genome sequence is located at the C-terminal DDE (Asp, Asp, Glu) motif. It binds to the IRs of *SB* transposon in a substrate-specific manner, and mediates a precise "cut-and-paste" transposition in vertebrate cells (**Fig. 1B**).[13]

SBase recognizes the IR/DRs terminals of the transposon, excises the transposon, and facilitates its insertion into targeted chromosomal DNA through NLS, a "cut-and-paste" process.[10,14] This process can be divided into 5 major steps: (i) specific binding of SBsase to designated sites within the transposon IR/DRs; (ii) pairing of a synaptic complex within 2 ends of the elements and binding together by SBsase subunits; (iii) excision from the donor locus; (iv) recognition of the target sequence in genome; and (v) reintegration at the target locus (**Fig. 2**).

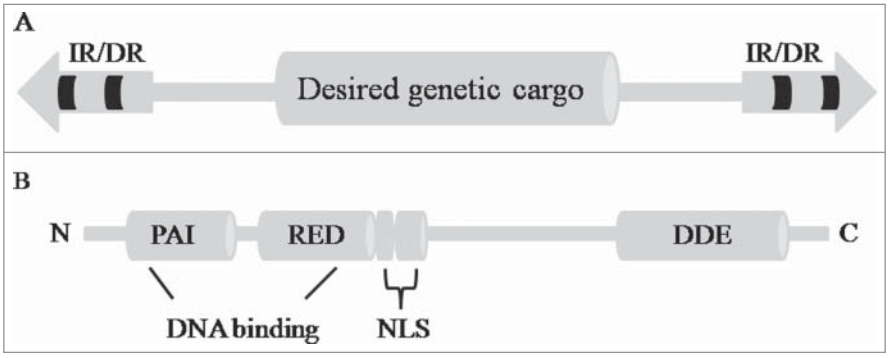

Figure 1. The structures of *SB* transposon and transposase. (A) The transposon has a desired genetic cargo, which is flanked by terminal inverted repeats (IR/DRs, 2 big arrows), each containing 2 binding sites for the transposase. (B) *SB* transposase has an N-terminal, bipartite, paired-like DNA-binding domain [PAI (Pro, Ala, Ile), RED (Arg, Glu, Asp)] containing a nuclear localization signal (NLS); and C-terminal, which has the DDE (Asp, Asp, Glu) catalytic domain.

Different subtypes of *SB* transposon system

To enhance the transposition efficiency, hyperactive versions of *SB* transposon system have been developed *via* modifying SBase or the transposon IR coding region.[10-12,15,16] For example, several modified versions of SBase including SB10, SB11, SB100,[17,18] and SB100X with increasing catalytic activities have been developed. SB100X is up to 100 times more active than the original one, displaying the highest efficiency (24%) compared with SB11 (1.23%) and *Piggyback* (3.8%).[19]

However, the transposition efficiency decreases sharply if the inserted sequence is more than 4 kb in length. To solve this problem, a biologically mimic *SB* transposon system has been developed. It contains a gene of interest flanked by 2 mutant *SB* transposon elements in an inverted orientation. Since each single mutant *SB* transposon element contains CA to GC mutations at the terminus of the right IR/DR domain, the induced mutations interfere only with the catalytic steps of transposition but not with SBase binding. As a result, the new *SB* transposon system has superior ability to transpose genes of >10 kb in length.[11]

In addition, a cyclization recombination enzyme (Cre) inducible SBase allele, *Rosa*SBaseLsL, which allows the restriction of transposon mutagenesis to a specific tissue of interest, has been established to facilitate the insertion of genes to specific tissue (s).[20] Since mammalian genome lacks high affiliative *lox*P sites, so Cre/*lox*P system can be effectively applied in mammalian system without confusing with its own system.[21,22] The temporal and spatial Cre recombinase expression in mammalian has been well established.[23-26] Thus, this system can be applied to transpose gene(s) of interest to specific tissue(s).

Advantages of *SB* transposon system

SB transposon system becomes popular in mammalian gene transfer due to following reasons. First, the ability of SBase to distinguish its own substrate from very similar sequences and the correction process during synaptic complex formation period make the *SB* transposon system enable to accurately recognize IR/DRs and catalyze the right substrate.[13] Second, *SB* transposon system is relatively safe for transposition. Studies have shown that neither SBase nor *SB* transposon has remarkable toxicity in mice.[27-30] The effective expression of SBase stops 4 days after hydrodynamic injection.[31] Third, the expression of integrated genes *via* *SB* system is long-term and reliable, even pass to next generation *via* germline transmission.[32] Fourth, *SB* transposon system can integrate in various tissues including human cells, one-cell mouse embryo, mouse embryonic stem cells, and mouse somatic tissues. It can theoretically integrate in more than 340 million TA sites, the target sequences for *SB* transposon system in mouse genome.[32-41] Although both *Piggyback* and *SB* transposon systems display bias toward integration in actively transcribed loci, *SB* transposon system can integrate within a

wide region of 4 Mb near the donor locus.[42] Fifth, with the help of *Rosa*SBaseLsL, SB system can target or integrate genes in given tissues.

Disadvantages of *SB* transposon system

Nevertheless, there are some disadvantages of *SB* transposon system. First, overall size of the transposon vector and the ratio of SBase to transposon would affect transposition efficiency, a phenomenon that occurs in *SB* but not observed in *Piggybac*.[43,44] However, this limitation can be circumvented by that the *SB* transposon and SBase are transfected with different vectors or that SBase is provided in the form of either mRNA or protein.[38,45,46] Second, *SB*-mediated integrated gene(s) might be transcriptionally silenced in mammalian cells.[47,48] Gene-regulatory domain at the terminal of *SB* may produce complementary RNAs by RNA interference response against the transposon.[49,50] The addition of 2 heterologous 5'-HS4 chicken β-globin insulators between genetic cargo and the IR/DR domain may prevent the transcriptional silencing of *SB* transposon system, thus improving the transposition efficiency.[47] Third, due to the "cut-and-paste" mechanism, the *SB* transposon ends may not be excised, leaving a "footprint" mutation–a 5-bp insertion mutation containing a TA element.[51-55]

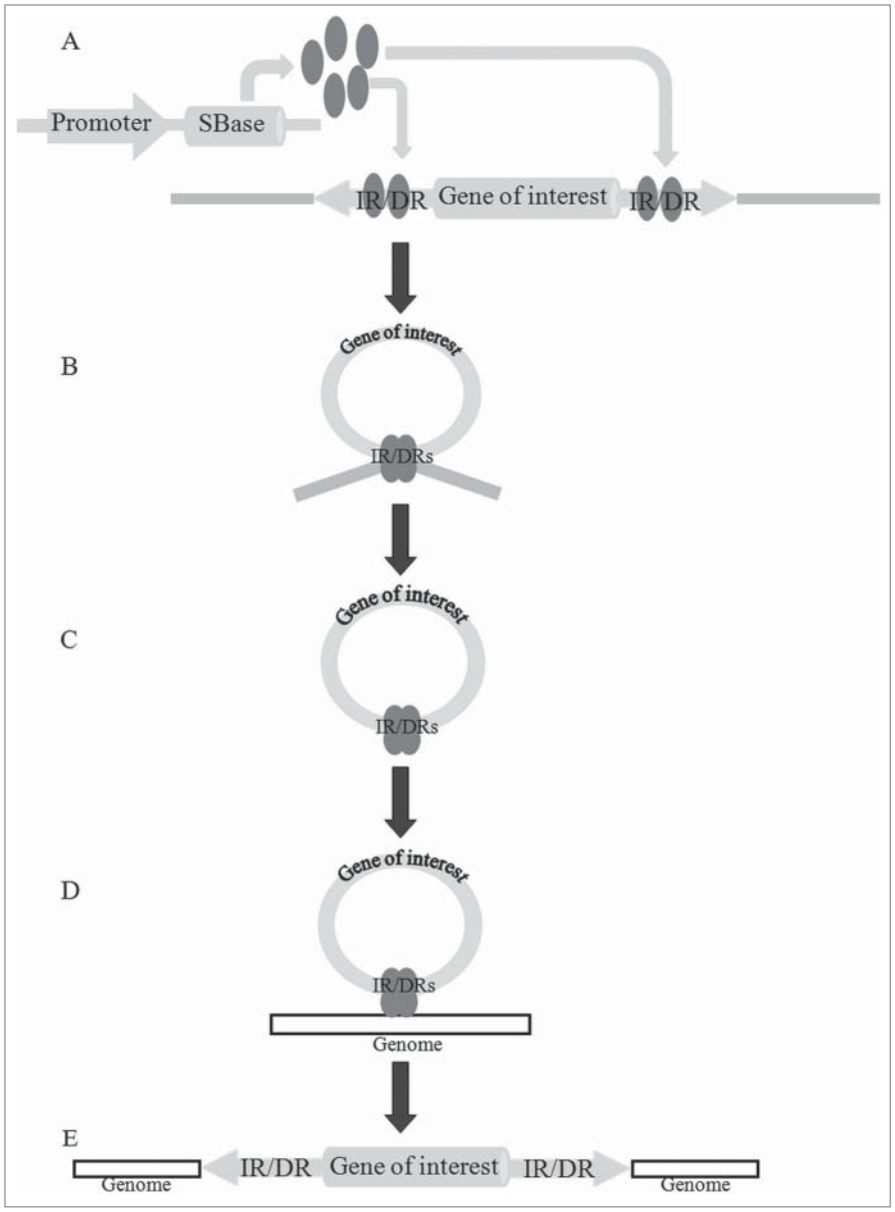

Figure 2. The "cut-and-paste" process of integrating gene(s) of interest into host genome. (A) SBase, whose expression driven by the promoter, recognizes the IR/DR sequence of the transposon and binds to these sites. (B) The synaptic complex formatted. (C) SBase tetramers cut the donor sequence between IR/DR sites. (D) SBase tetramers recognize target sites in the genome and bind to it. (E) The gene(s) of interest between IR/DR sites reintegrate into the genome.

The Application of *SB* Transposon System for Genetic Etiological Research and Gene Therapy of Cancers

***SB* transposon system to construct cancer models**

Animal models are frequently used in genetic etiological research of cancers. Tumorigenesis can be induced *via* overexpressing oncogenes and/or down-regulating tumor suppressor genes in animal models. Thus, mammalian models of cancers can be obtained by integrating or targeting specific genes in the genome of animals.[56] Transposition using the *SB* system provides a novel method to construct cancer models.

There are several approaches for *SB* transposon system to induce tumorigenesis in animals. First, as *SB*-mediated integrated gene can be transmitted to next generation, it is relatively easy to generate and maintain whole libraries of integrated mutants in the founder animals. Tumors can be observed and analyzed *via* breeding the founders, skipping the process of classical embryonic stem cell–germline chimera–mutant.[20,29,30,57-59] Second, combination of SBase with T2/Onc2 or T2/Onc3 transposon can randomly elicit mutations that result in different types of cancer. T2/Onc2 transposon contains the 5' long terminal repeat (LTR) of murine stem cell virus (MSCV). T2/Onc3 is identical to T2/Onc2 other than replacing the MSCV LTR with CAG promoter, which consists of cytomegalovirus (CMV) enhancer/β-actin promoter.[20] Both transposons can elicit overexpression of nearby proto-oncogenes. SBase can help T2/Onc

insert into host genome to activate proto-oncogenes. The 5'LTR in T2/Onc2 usually drives the expression of proto-oncogenes at higher rates in haematopoietic cells than in cells of other histotypes.[20] CAG promoter in T2/Onc3 is active in a variety of cell types, including epithelial cells.[20] In addition, the *Rosa*SBaseLsL can express SBase depending on the expression of Cre gene. A variety of carcinomas can be induced in different tissues based on different *SB* transposon systems.[20,29,30,57-61] For example, over 20 different types of cancers have been induced using SBase with T2/Onc3 in mice.[20] Triple transgenic (*Rosa26*-lsl-SB11; T2/Onc; albumin promoter-driven Cre) and quadruple transgenic (*Rosa26*-lsl-SB11; T2/Onc; albumin promoter-driven Cre; p53-lsl-R270H) mice partially generate liver tumors displaying hepatocellular carcinoma (HCC) characteristics and lung metastasis at late stage.[29] Similarly, by using *Villin*-Cre to activate SBase expression in gastrointestinal epithelium specifically, the transgenic mice can develop intraepithelial neoplasia, adenocarcinoma and adenoma.[30] With the use of bovine keratin K5 promoter to drive SB11 expression in epidermal stem cells, the transgenic mice (K5-SB11 and T2Onc2) are more likely to generate papilloma, squamous cell carcinoma, and basal cell carcinoma of the skin than the wild-type counterparts after 7,12-dimethylbenzanthracene/12-O-tetradecanoylphorbol-13-acetate (DMBA/TPA) or only TPA treatment.[57] *SB* transposon system harboring the T2/Onc element can facilitate tumorigenesis compared to controls without this *SB* transposon system.[58,59] T2/Onc3 is more powerful than T2/Onc2 in inducing carcinogenesis.[59] In addition, different genetic background can influence tumorigenesis in mice. On the $Ptch^{+/-}$ background, the transgenic mice with T2/Onc transposon and cerebellar progenitor cells-specific expressed SB11-transposase driving by *Math1* promoter have an increased progression of medulloblastoma. On $Tp53^{mut}$ ($Tp53^{+/-}$ or $Tp53^{-/-}$) background, this *Math1*-SB11/T2Onc transposon system facilitates the development of disseminated medulloblastoma.[61] Third, replacement of T2/Onc with specific oncogene, *SB* transposon system can induce specific oncogene-driven carcinomas. For example, through hydrodynamic tail vein injection, *SB* transposon system containing an activated *N-RAS* oncogene can elicit multifocal liver cancer in *p19Arf*-null or heterozygous mice.[62] Subcutaneous injection of *SB* transposon system harboring oncogenes, including *c-Myc, H-RAS,* and short hairpin RNA against the transformation related protein 53 (*Trp53*) gene (*shp53*), into female C57BL/6 mice can induce sarcomatoid carcinomas in skin (**Table 1**).[63] The *SB* system can also be applied in other mammalians, such as rats. Single gene transgenic rats can be interbred to obtain double-transgenic rats.[64] With the help of electroporation method, *SB* transposon system can also deliver *c-Myc, H-RAS*, and *shp53* oncogenes into rats to produce liver tumor.[65] In addition, the SB100X transposon system has been ever used for enzyme-catalyzed gene integration into the embryonic porcine genome.[66] Thus, *SB* transposon system can be applied to construct a variety of animal models for genetic etiological research of cancers.

Table 1 *SB* transposon system used for the construction of cancer mouse models

Transposon insert element	Cancer type	Method	Animals	Ref.
T2/Onc3	Squamous cell carcinoma, HCC	Pro-nuclear injection, knock in ES cell technology, ES cell electroporation, hybridization	C57BL/6J mice and C57BL/6J C3H hybrid mice	20
T2/Onc	HCC	Pro-nuclear injection, knock in ES cell technology, hybridization	Hepatocyte-specific *Alb*-Cre mice, Rosa26-lsl-SB11 mice and *p53*-lsl-*R270H* mice	29
T2/Onc2	Intestinal intraepithelial neoplasia, adenocarcinoma, and adenoma	Pro-nuclear injection, knock in ES cell technology, hybridization	*Rosa26*-lsl-SB11 mice, *Villin*-Cre mice, and T2/Onc mice	30
T2/Onc2	Skin cancers	Pro-nuclear injection, hybridization	C57BL/6J × DBA/2J F2 embryos, AC heterozygous mice	57
T2/Onc	Liver cancer	Pro-nuclear injection, knock in ES cell technology, hybridization	Tet-on-*MYC* mice, LAPtTA mice, *Rosa26*-SB11 mice, and T2/Onc mice	58
T2/Onc2 and T2/Onc3	Pancreatic adenocarcinoma	Pro-nuclear injection, knock in ES cell technology, hybridization	LSL-$Kras^{G12D}$, *Pdx1*-Cre, T2Onc2, T2Onc3, and *Rosa26*-LSL-SB11 transgenic mice	59
N-RAS	liver cancer	Hydrodynamic tail vein injection	C57BL/6J *p19Arf*-null mice	62
H-RAS, *c-Myc*, *shp53*	Sarcomatoid carcinoma	Subcutaneous injection	C57BL/6 female mice	63
Fah, *HBx*, *shp53* and *N-RAS*	HCC	Hydrodynamic tail vein injection	*Fah* $^{-/-}$ mice	72

Alb, *Albumin*; *c-Myc*, v-myc avian myelocytomatosis viral oncogene homolog; CRC, colorectal cancer cell; Cre, a cyclization recombination enzyme; ES cell, embryonic stem cell; *Fah*, fumarylacetoacetate hydrolase gene; HCC, hepatocellular carcinoma; *H-RAS*, Harvey rat sarcoma virus oncogene; *Kras*, Kirsten rat sarcoma viral oncogene homolog; LAPtTA, a liver-specific tet-transactivator protein; lsl, *lox*P-stop-*lox*P; *N-RAS*, neuroblastoma ras oncogene; *p19Arf*, a positive regulator of the p53 tumor suppressor, and loss of Arf predisposes to a wide spectrum of tumors; Pdx1, pancreatic and duodenal homeobox 1; *p53*-lsl-*R270H*, *R270H* targeted point mutations of *p53*, which is considered as a conditional dominant negative *p53* transgene; *Rosa26*-lsl-SB11, SB11 transposase cDNA preceded by lsl knocked into the *Rosa26* locus; tet-o-*MYC*, tetracycline-repressible *MYC* transgene; *shp53*, a short hairpin RNA against tumor suppressor *Trp53* encoding gene.

SB transposon system for the discovery of cancer driver mutants

GWAS, microarray, and deep sequencing for cancer gene discovery are carried out *via* comparing the differences between cases and controls or between cancerous tissues and non-cancerous tissues from the same individual.[67-71] These designs are hard to distinguish driver mutations from passenger mutations because cross-sectional case-control studies can only indicate statistical associations between factors of interest with the diseases. If loss-of-function or gain-of-function mutations can promote carcinogenesis, these mutations are more likely to be driver mutations. *SB* transposon system can introduce gain-of-function mutations, such as combining with T2/Onc to cause mutations randomly or deliver some oncogenes or tumor suppressor genes. On the other hand, loss-of-function mutations can be achieved by using *SB* transposon system to deliver specific elements to silence genes, such as *shp53*.[72] Thus, *SB* can be used to distinguish driver mutations from passenger mutations. Here, we introduce several examples. To identify genetic drivers of malignant peripheral nerve sheath tumor (MPNST), the *SB* transposon system has been used to characterize mutations in mice based on the following steps. First, *SB* expression and activity are confirmed by immunohistochemistry and PCR-excision assay, respectively. Second, common insertion site (CIS) analysis is utilized to identify potential driver-mutations by both TAPDANCE CIS and gene centric CIS analysis. Third, relevance of the CIS-associated genes to MPNST is evaluated by cross-species comparative analysis of the CISs to previously generated human array comparative genomic hybridization, SNP array, human gene expression profiling, and methylome data from normal Schwann cells, neurofibromas, and MPSNTs. Fourth, Ingenuity Pathway Analysis, Database for Annotation, Visualization and Integrated Discovery are utilized to identify significantly altered signaling pathways in CISs including Wnt/CTNNB1, PI3K/Akt/mTOR, and growth factor receptor signaling pathways. Last, further validation of novel candidate driver-mutations, like *Foxr2*, is performed by over expression and knockout experiments.[60] The second Nebulin family member, *NEBL*, is involved in *MLL* gene rearrangement, a phenomenon frequently observed in infant acute myeloid leukemia. Stable transfection of *SB* transposon system harboring the expression cassettes for *MLL-NEBL* and *NEBL-MLL* has demonstrated that the fusions have oncogenic potential.[73] In *SB* transposon system–induced mouse models, retrotransposon-like 1 (*Rtl1*) and *PDE4D* have been identified as drivers of HCC and prostate cancer, respectively.[74,75] *MYC* is a dysregulated gene in human malignancies. Introduction of *MYC* through *SB* transposon system can generate liver cancer in a mouse model. Genetic screening and functional validation studies in this model have shown that *Ncoa2/Src-2* is a tumor suppressor gene in liver cancer.[58] *MyoD* is a well-known muscle differentiation factor. A recent *SB* transposon screening study has shown that its expression in cerebellum hinders the development of medulloblastoma, providing further evidence that *MyoD* is a tumor suppressor gene for medulloblastoma.[76] The *SB* transposon system can also be utilized to identify genes critical in tumor dissemination. Functional genomics has demonstrated that ectopic expression of *Eras*, *Lhx1*, *Ccrk*, and *Akt* are associated with Sonic Hedgehog signaling induced dissemination process of medulloblastomas in Patched$^{+/-}$ mice.[77]

SB transposon system for cancer gene therapy

With the continual discovery of genes and genetic mutants that promote cancer development, gene therapy becomes more and more practicable options for cancer treatment. *SB* transposon system can be implicated in cancer gene therapy.

Viral-based gene delivery is a preferred choice for gene transfer, but it has several limitations. First, viral vectors are very likely to elicit immune/inflammatory or neurotoxic responses that are associated with contamination during bacterial extraction process. Second, viral preparations impose risks of contamination by infectious factors such as endogenous proviruses, or replication-competent viruses. Due to their tendencies to integrate near promoters or transcriptional units, viral vectors may cause unwanted cellular consequences. Third, it is relatively costly and time consuming of viral preparation. To overcome these limitations, *SB* transposon system is an alternative for cancer gene therapy. For example, an engineered *SB* transposon system coexpressing a single-chain chimeric antigen receptor (CAR) for human CD19 and CD20 has been used to integrate into the chromosome of T cells from peripheral blood mononuclear cells (PBMNCs) and umbilical cord blood. Stable dual-gene expression in T cells from PBMNCs and umbilical cord blood allows for the enrichment by positive selection with Rituxan. Both CD4$^+$ T cells and CD8$^+$ T cells can display the cytotoxicity against CD19$^+$ leukemia, lymphoma, and erythroleukemia cell lines and release high-levels of antigen-dependent Th1 (but not Th2) cytokines, like granulocyte-macrophage colony-stimulating factor, TNF-α, and IFN-γ. In animal experiments, these engineered T cells significantly decrease tumor growth and increase survival time.[78] Following the similar procedures, CD19-CAR-specific T cells,[79-81] *MART-1* and *p53* targeting PBMNCs[82] and IL-11Rα-CAR-specific T cells[83] constructed using *SB* transposon system show optimal effects in treating lymphoid malignance or osteosarcoma pulmonary metastases. In addition, *SB* transposon system is able to directly transfer therapeutic genes *in vivo* for cancer treatment. A modified *SB* transposon system containing an angiostatin-endostatin fusion gene (*StatinAE*) has been applied in the CT26 mouse model of CRC metastatic to the liver. This study has demonstrated that this *SB* transposon system is effective in treating metastatic CRC.[84] *SB*-mediated insertions of each of *StatinAE* and a soluble vascular endothelial growth factor receptor are proven to be effective for the treatment of glioma.[85] The same is true for the *SB*-mediated insertion of suicide gene herpes simpex virus thymidine kinase controlled by human telomerase reverse transcriptase promoter and a SV40 enhancer for the treatment of HCC.[86] Gene transfer mediated by *SB* transposon system, can improve the efficacy of immune gene therapy *via* sustaining cytokine secretion and direct intratumoral delivering of DNA/polyethylenimine complexes of mIFN-γ/SB construction (**Table 2**).[87]

Table 2. *SB* transposon system used for cancer gene therapy

Insert gene	Cancer type	Experimental animals or cells	Method	Ref.
CAR for human CD19 and CD20	$CD19^+$ lymphoid malignancies	PBMNCs and umbilical cord blood T cells; (NOD/SCID) mice irradiated and injected intraperitoneally with Daudi-LVhfflucN	Transfection; infusion *in vivo*	78
CAR for human CD19 and CD28	$CD19^+$ lymphoid malignancies	Daudi (Burkitt lymphoma), HLA^{null} K562 (erythroleukemia) cells and human PBMCs	Transfection	79
CD19RCD28 transgene	B-lymphoid malignancies	K562 cells and PBSC	Electroporation	80
CAR for human CD19 and CD28	$CD19^+$ lymphoid malignancies	PBMCs from healthy adult volunteer donors, CLL cells, MCL cells, diffuse large B-cell lymphoma cells	Electroporation	81
p53 TCR and anti-*MART-1* TCR	Cancer and immunologic disease	PBMNC	Electroporation	82
IL-11Rα-CAR	OS lung metastases	Human T cells, K562, Human OS cell lines (CCH-OS-D, KRIB, SAOS-2 and LM7)	Co-electroporation	83
StatinAE	CRC metastasized to liver	BALB/c female mice with intrasplenically transplanted CT26 colorectal tumors	Hydrodynamically injected	84
sFlt-1 and *statinAE*	Glioma	Nude mice with GBM xenografts (subcutaneous injection of U373 or U87 cell lines)	Injection	85
Suicide gene *HSV-TK*	HCC	HepG2, Hep3B, Huh7, and hNHeps cell lines	Transfection	86
INF-γ	Glioblastoma	GL261 cells and C57BL/6 mice	Transfection; slow injection into the skull	87

CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukemia; CRC, colorectal cancer; GBM, glioblastoma multiforme; HCC, hepatocellular carcinoma; HLA, human lymphocyte antigen; HSV-TK, herps simplex virus thymidine kinase; IL-11Rα-CAR, Interleukin-11 receptor α-chain CAR; *INF-γ*, interferon gamma gene; MCL, mantle cell lymphoma; OS, osteosarcoma; PBMCs, peripheral blood mononuclear cells; PBSC, peripheral blood stem cell; sFlt-1, soluble vascular endothelial growth factor receptor; *StatinAE*, an angiostatin-endostatin fusion gene; TCR, T-cell receptors.

Furthermore, the first clinical trial of *SB*-mediated gene therapy was initiated at MD Anderson Cancer Center in 2013. After modifying T cells collected from patients or from matched donors, CD19-CAR-specific T cells were then used to treat patients with leukemia or lymphoma. No acute and late toxicities were found and one of the first 5 treated patients remained in remission.[88]

SB transposon system in Hepatitis B Virus (HBV) integration

SB transposon system had been applied in elucidating the mechanism of HBV-induced carcinogenesis in animal model. With the use of hydrodynamic delivery method, *SB* transposon system can introduce HBV X (*HBx*) gene into the livers of fumarylacetoacetate hydrolase (*Fah*) mutant mice and induce hepatic inflammation. Coexpression of *Fah* cDNA from the transposon vector allows for the selective repopulation of genetically corrected hepatocytes in *Fah* mutant mice. The subsequent selective repopulation of hepatocytes carrying the gene(s) of interest could provide useful genetic information about the mechanisms of HBV-induced neoplasm. In this mouse model, introduced HBx can activate the expression of β-catenin and HBx coinjected with *shp53* accelerates the formation of liver hyperplasia. Constitutively active v-ras oncogene homolog with Gly12Val substitution ($NRAS^{G12V}$) alone and in combination with *shp53* coinjection facilitate hepatic tumorigenesis.[72] Thus, *SB* transposon system can be applied to investigate the oncogenic effects of viral genes.

Conclusion

SB transposon system is a reliable tool to integrate mutations into mammalian cell genome and transmit the genes (or mutants) of interest to next generations. In combination with Cre, *SB* transposon system allows for the activation of introduced genes (or mutants) in specific tissue(s). *SB* transposon system theoretically reintegrate in more than 340 million TA sites in mouse genome, which can be applied in systemically screening for cancer-related genes. It can distinguish the driver mutations from passenger mutations and also serve as a delivery toolkit in inserting therapeutic genes into host genome to adjust the imbalances

between oncogenes and tumor suppressor genes. *SB* transposon system can combine different therapeutic approaches to improve cancer treatment. It has been applied in clinical trial for the treatment of leukemia and lymphoma. Although *SB* transposon system has a number of advantages, it might activate carcinogenesis by unwanted integrations. Furthermore, its safety and duration of gene expression are still uncertain. More researches are needed to improve and optimize the *SB* transposon system for clinical cancer gene therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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