Review Article

Novel Trends in Genetics: Transposable Elements and Their Application in Medicine

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Abstract

Forty-five percent of the human genome is composed of Transposable Elements (TEs); therefore, TEs have had an undisputed impact on evolution of the most evolved creature by a very simple mechanism of action. Scientists have been studying this simple mechanism of action and are currently using it to develop efficient and safe gene delivery systems especially for treatment of diseases. TEs have also been used safely in generating induced Pluripotent Stem Cells (iPSC) for regenerative medicine, which opens the door to a world of possibilities in our approach in trying to wrestle with many challenges in medicine. The PiggyBac (PB) system has yielded more success in generation of induced pluripotent stem cells in regenerative medicine, and the Sleeping Beauty (SB) has been more successful in Gene Therapy. Recent advances are indicative of more good news to come regarding the potential heights of successes achievable by the use of the TE-based systems.

Keywords: Gene therapy, genetic transposition, induced pluripotent stem cell (iPSC), transposable elements (TEs), transposable element based vectors (TEV)

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Introduction

Transposable Elements (TE), also known as "jumping genes," compose forty-five percent of the human genome (Figure 1) which had previously been considered as junk or selfish DNA. Only recently scientists are discovering the real impact of TEs in re-structuring genomes and in evolution. In the 1950s, Barbara McClintock was the first geneticist to note mobile elements in maize. While doing classical genetic experiments, she proposed that TEs can move within and between genomes and suggested them as "controlling elements" which in 1983 resulted in her unshared noble prize in physiology or medicine for genetic transposition.[1] Later in the 1970s, *P* elements and *I* elements were shown in fruit fly *Drosophila melanogaster* to lead to hybrid dysgenesis phenomenon.[2–5] In addition, TEs present in bacteria are responsible for delivering antibiotic resistance genes.[6] The evolved mammalian genome contains plenty of repetitive sequences mostly derived from TEs. These findings alongside other discoveries demonstrate the remarkable role of TEs in the structure, function and evolution of various genomes. It is obvious that in the 1950s, no one could have imagined the significance of TEs in genetics, but in her 1992 book, the Dynamic Genome, Barbara McClintock said, "I believe there is little reason to question the presence of innate systems that are able to restructure a genome." It has become increasingly clear that TEs not only serve a key role in genome evolution but also could have great utility in gene delivery systems as vectors, especially in gene therapy, by monopolizing their simple but clever mechanism of action.

TE vectors are promising delivery systems designed after the natural model of TE mechanism of action in genomes.[8] Indeed, the evolutionary process of gene therapy vectors began with plasmids and naked DNA as the first generation vehicles, followed by viral vectors as the second generation vehicles, and has recently progressed to transposable element vectors (TEV) as third generation vehicles. Each of these vectors has advantages and disadvantages, which should be evaluated with consideration given to nature of the target Gene of Interest (GOI), cell, tissue, and organism. Among these, viral vectors are most commonly used for gene therapy application; however, the third generation TEVs are competing with viral vectors in gene therapy. The first TEV is *Sleeping Beauty* (*SB*) which evolved by molecular technologies to become more suitable for therapy.[9] In fact, it should be noted that there are several gene therapy methods being produced, such as nanoparticles and exon skipping, which should also be considered in respect to each therapeutic.[10] These are all targeted therapy strategies with potential for prospective patients.

In this review, TEs will be briefly reviewed with discussion of different types of TEs and their mechanism of action. Then we will describe implications of TEVs in gene therapy and iPSC production in comparison with common gene delivery vectors.

Transposable elements and mechanism of transposition

Transposon elements are interspersed repeats composed of very large numbers of copies of relatively few sequence families and contribute to 45% of the human genome (Figure 2), whereas protein-coding regions just comprise 1.5% of the human genome.[11]

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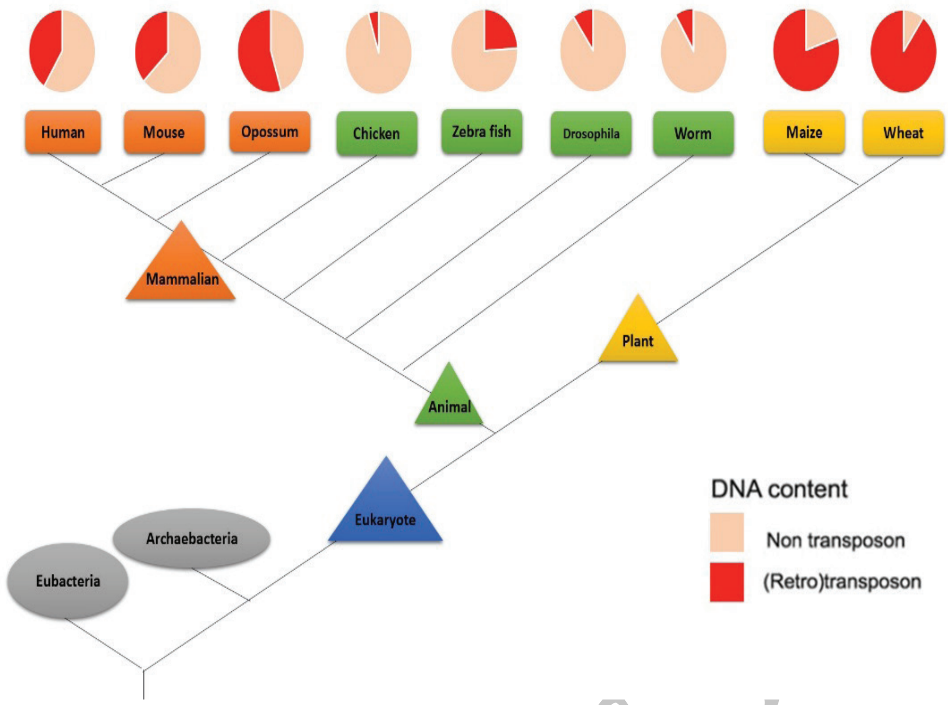
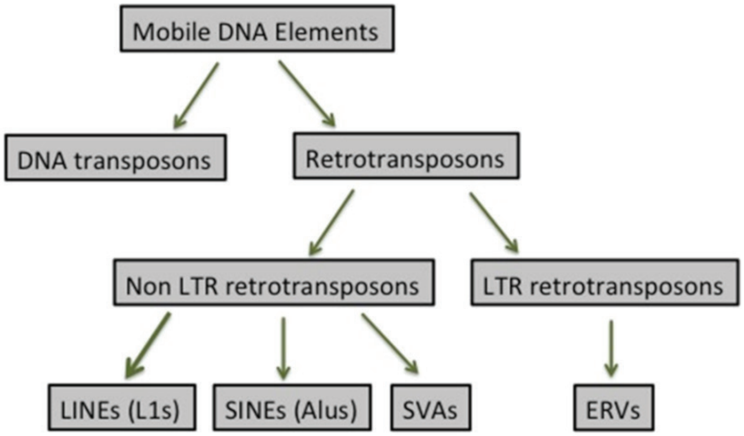


Figure 1. Phylogenetic tree of life. Organisms are shown with the fraction of their genome made-up of transposable elements.[7]

a. Transposable element classes



b. Transposable element in human genome

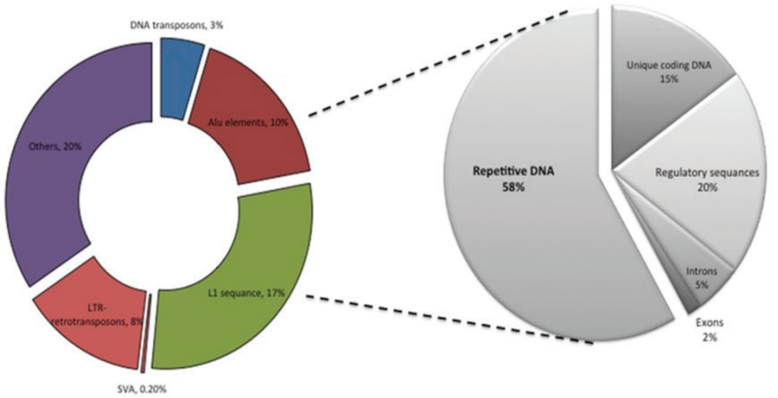


Figure 2. Transposable Elements. **a)** Transposable Element classes, **b)** Transposable Elements in human genome.

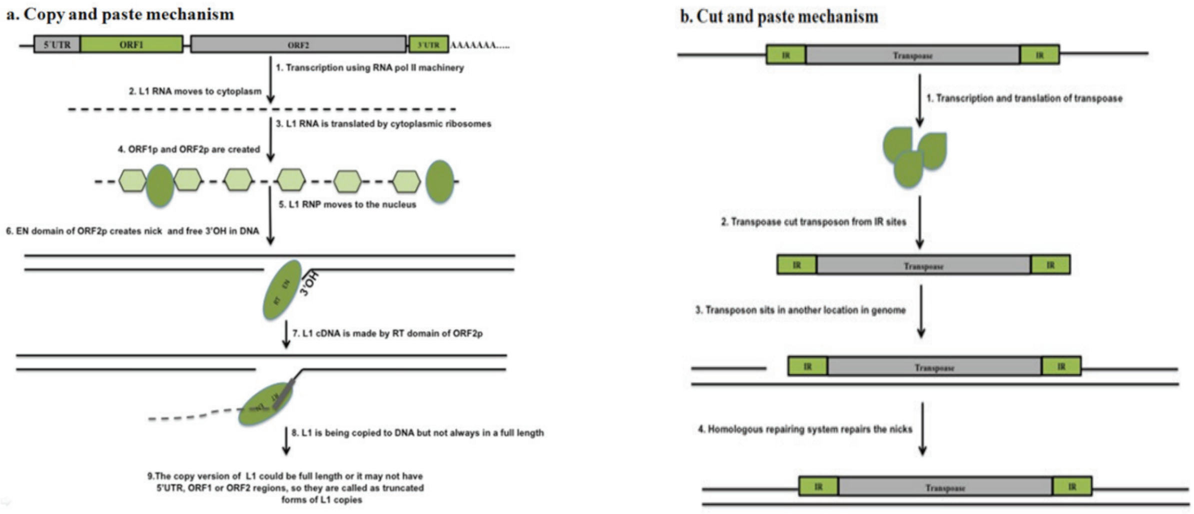


Figure 3. Transposition mechanism, **a)** retrotransposition, **b)** DNA transposition.

In the human genome, TEs contain two major classes: DNA-based TEs (DNA transposons) with cut-and-paste mechanism and RNA-based TEs (retrotransposons) with copy-and-paste mechanism (Figure 3).[12]

Three percent of the human genome consists of DNA transposons which use a cut-and-paste mechanism for mobilization within the genome. They excise themselves from the genome and move, then insert into another region in the genome by transposase activity (Figure 3b).[13] DNA transposons were active in primate evolution but they do not currently have mobile activity in the human genome.[14]

Retrotransposons use a copy-and-paste mechanism to insert into a region in the genome. In this mobilization mechanism, the retrotransposon is transcripted into RNA, and then reverse transcriptase synthesizes DNA from an RNA product. It is the DNA product which inserts into the genome (Figure 3a). Retrotransposons, based on presence or absence of long terminal repeats, subdivide into two groups: LTR and non-LTR. Eight percent of the human genome consists of human LTR elements that are endogenous retroviruses (HERVs). LTR retrotransposons are derived from full length proviral DNA by homologous recombination between the two LTRs. They are rarely active in humans.[15] Non-LTR retrotransposons compose approximately one-third of the human genome; therefore, the majority of human TEs result from the present and past activity of these elements. Non-LTRs include three types of elements: SINE, SVA and LINE.[11,16–19]

Non-LTR elements are active in the human genome and result in at least 60 genetic disorders due to *de novo* insertional mutations.[20,21] It has been recognized that recombination between TEs can cause genomic deletions which cause several genetic disorders.[22] Overall, TEs can contribute to genetic variations, polymorphisms and alter gene expression.[23]

LINEs in the human genome consist of three major families (L1, L2, and L3) which differ in their sequence. L1 elements constitute around 17% of the human genome. L1 is 6 kb long with >500,000 copies in the human genome.[11] Less than 100 copies are functional, because most L1 copies are inactivated by mutations, truncations and internal rearrangements.[24,25] L1 is the only active autonomous TE in the human genome. L1 with retrotransposition multiplies itself in the genome.[26]

The Alu element is in the SINE group. Alus compose 10.6% of the human genome and have more than one million copies in it.[26] This high number of copies is due to past continuous mobilization activity. Alu length is ~300bp. Alus are non-autonomous TEs, don't have a coding region and use the retrotransposition machinery of L1's. The SVA element is made up of a SINE (Short Interspersed Element) region, a VNTR (Variable Number Tandem Repeats) region and an Alu-like region. They are ~2 kb long with ~300 copies in the human genome. SVA elements, like Alu's, are non-autonomous and use the L1 retrotransposition mechanism system (Figure 4).[27,28,19] There are old, inactive non-LTR retrotransposon families other than L1, Alu and SVA that comprise ~6% of the human genome.[29] Despite L1, Alu, and SVA which are currently active, these old families are inactive now and provide a rich molecular "fossil record" that affirm the long relationship between the human genome and the TEs.[11]

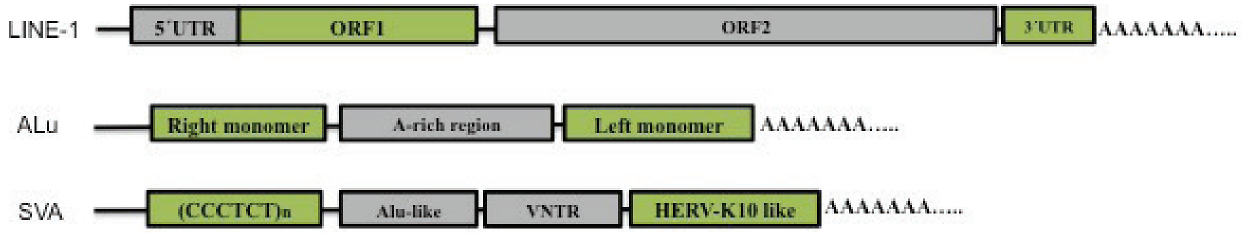


Figure 4. Structure of L1, Alu, SVA.

Transposable elements to ameliorate human diseases

DNA transposons are excised from a donor locus and then integrated into another location (cut-and-paste mechanism) by a transposase. This mechanism is a key feature in using DNA transposons as gene delivery systems. Transposase works via a cut-and-paste mechanism in *trans* for any DNA sequence that is flanked by the terminal repeat sequences required for transposition. A binary system (*trans*) has been developed for turning the DNA transposons into a gene delivery tool which is composed of two plasmids: an expression plasmid coding a transposase and a donor plasmid containing the DNA of interest to be integrated, which is flanked in *cis* by the transposon terminal repeat sequences required for transposition. The transposase recognizes these terminal repeats, binds to it, and then by a cut-and-paste mechanism cuts GOI from the donor plasmid and inserts it into the host genome. This system uses two plasmids to physically separate the transposase gene from the transposon vector GOI. Thus it is possible to optimize the stoichiometry of both components. It is also possible to place the transposase gene in the same plasmid (donor plasmid) but outside of the terminal repeat sequences (*cis*).[30] In addition, it is possible to insert the mRNA of transposase into the engineered cells instead of insertion of the transposase gene in an expression vector during transposition.[30]

DNA transposons, as a tool, can act in germline transgenesis and insertional mutagenesis in invertebrate genomes. Several Tc1/mariner transposons (DNA transposons) have been isolated from insects and nematodes and have some activity in vertebrate genomes but don't have sufficient activity in mammalian genomes; therefore, their use is limited for gene therapy. For gene therapy, we should identify TEs capable of efficient transposition in mammalian cells. This goal can be achieved by molecular reconstruction of natural transposons. Moving in this direction, scientists have produced a synthetic and active Tc1/mariner type transposon named *Sleeping Beauty* (*SB*). To the best of our knowledge, until now three transposon vectors have been produced for gene therapy: *SB, Tol2,* and *piggyBac* (*PB*).[9,31,32]

Transposable Element based vectors (TEV)

Sleeping Beauty (SB) elements

SB was produced from combining fragments of defective and silent Tc1/mariner elements from salmonid fish and an ancestral transposon which had become inactivated during evolution.[9] Reconstructed *SB* had appreciable transposition efficiency in vertebrate cells at that time. The re-derived, reconstructed *SB* transposon had at least 10-fold higher efficiency than nature's Tc1/mariner transposons.[33] The limitation of mariner transposon family includes *SB* "overproduction inhibition" (OPI)-transposition efficiency decreases in presence of excess transposase activity. Therefore, the transposase-to-transposon ratio should be optimized.[34] SB has had wide implications for somatic gene therapy, transgenesis, insertional mutagenesis, and functional genomics.[8] The resurrected *SB* was not sufficiently robust for human gene therapy.[35] The main challenge for transposon vectors is the enhancing transpositional activity. Use of *in vitro* techniques could allow derivation of novel engineered *SB* transposases (*SBase*) with relatively modest increase in transposition activity. *SB100X* is the most hyperactive transposase engineered.[36–39] SB100X has ~100-fold enhancement in efficiency in mammalian cells compared with the originally resurrected *SB* in mobilizing a transposon for integration into the genome.[40]

Tol2 elements

Tols belong to a naturally occuring hAT superfamily. *Tol2* is a fish transposon that shows activity in human and most vertebrate cells.[31] *Tol2* can transfer genes up to 11 kb with minimal loss of transposition and this is an advantage.[41] Therefore, *Tol2* can carry larger genes than Tc1/mariner family transposons. Also, *Tol2* doesn't show OPI phenomena seen in the mariner transposon because *Tol2* transposition is directly proportional to the level of transposase.[42] *Tol2* has low transposition activity in comparison with *PB* and hyperactive *SB* systems. Similar to other hAT transposons and *PB*, *Tol2* may have preference for insertion into genes.[43] By modifications at its N-terminus, *Tol2* transposition is abolished.[43] Therefore, it is a challenge to generate a site specific integrating *Tol2* as a safe tool. On the other hand, *Tol2* creates single copy insertion and does not cause large rearrangements around the integration site.[43]

PiggyBac (PB) elements

PB was first discovered when it transposed from its insect host (the cabbage looper moth Trichoplusia) into the baculovirus genome.[32] *PB* can catalyze transposition in human and mouse somatic cells.[43] *PB*ase's transposition activity is higher than an early-generation *SB*ase and *Tol2ase* but its activity is lower than the *SB100X* hyperactive transposase system.[40,42] Molecular evolutionary methods, similar to those used to produce *SB100X* hyperactive transposase, were used to increase the efficiency of *PB*. Recently, Burnight, et al., demonstrated that the novel hyperactive *PB*ase had higher transposition activity than previous *PB*ase with a different insertion pattern.[44] Both *SB* and *PB* are complementary transposon systems. It would not be correct to prefer one over the other because their efficacies and properties may vary depending on the size of the GOI, the targeted cell, and the targeting site in the genome.[8]

Viral vectors versus transposable element vectors

Over time, virus based vectors have become highly effective in infecting cells, inserting into genomes and expressing contained genes. Some viruses can integrate their genome into the host genome to provide long lasting transgene expression, but this can poses safety risks. On the other hand, some viruses cannot integrate into the host genome and remain in extra-chromosomal locations in cells and have transient expression. Viruses can insert into cells by recognition and binding to specific receptors on the host cell surface, while TEVs cannot penetrate the plasma membrane and usually nucleofection-based methods have been used for introduction of TEV into the nucleus which lead to inefficient integration compared to viruses. Some viruses infect broad range of cells which is known as broad tropism and others bind to receptors of only a few cell types (narrow tropism). Some viruses can potentially accept large DNA inserts. Some viruses that are used as gene therapy vectors are naturally pathogenic and can trigger activate immune responses in the host. Viral vectors such as gutless adenoviruses are made replication defective due to safety reasons. The γ-retroviral and lentiviral vectors are common viral vectors that have been used in gene therapy, which can integrate in the host genome. Adenoviral, adeno-associated and Epstein-Barr viral vectors also have been used as vectors but cannot integrate into the genome.[45]

Retroviruses have a single strand RNA as their genome and by reverse transcriptase/integrase activity, the resulting cDNA randomly integrates into the genome. γ-retroviral vectors are derived from avian retroviruses and simple mice and contain three transcription units: *gag*, *pol*, and *env*. γ-Retroviruses (e.g., Moloney murine leukemia virus [MLV]) have been used in all approved clinical hematopoietic progenitor cell gene therapy trials.[46] MLV derived γ-retroviral vectors prefer to integrate into transcribed genes and around promoters and CpG islands.[47] This indicates that these vectors can activate proto-oncogenes or silence tumor suppressor genes. These vectors transduce dividing cells only because γ-retroviruses cannot transmit their genome through nuclear pores.[45,48,49]

Lentiviruses have single strand RNA as their genome. These are complex retroviruses and most of these vectors are based on HIV (Human Immunodeficiency Virus). Lentiviruses can transduce dividing and non-dividing cells and their genome contains six early expression proteins before replication of the virus which also includes two regulatory proteins (*tat* and *rev*). These two proteins are essential and bind to specific sequences in the viral genome. They also contain late expression genes (*gag, pol*, and *env*). Lentiviral vectors strongly favor integration in actively transcribing genes but don't show any particular favor for promoter regions. Therefore, these vectors, like the previous one, pose risk of oncogenic activation.[45,50]

Adenoviruses have a double stranded DNA and can efficiently transduce both dividing and non-dividing cells. These viruses have relatively large genomes that cause unwanted severe immune responses.[45,51] Adeno-associated viruses (AAV) have a single stranded DNA genome and can insert into both dividing and non-dividing cells. They are non-pathogenic viruses and are unrelated to adenoviruses. Due to their reliance on co-infection by helper viruses (herpes or adenoviruses) for replication, these viruses were named adeno-associated viruses. These viruses have only two genes, *rep* and *cap*; *rep* encodes controlling viral replication, structural gene expression and chromosomal integration proteins, but *cap* makes capsid proteins. Adenoviruses have a usefully narrow tropism.[45,52] The AVV9 variant can be useful for spinal cord injuries because it has high tropism to the spinal cord and brain astrocytes.[53] The other advantage is exhibition of poor immunogenicity.[52] Herpes simplex viruses as complex viruses have double stranded DNA genomes with approximately 80 genes. They are highly tropic to the central nervous system and are non-integrating viruses with potential for long lasting expression.[45,54,55] Overall, because TEVs trigger less active immune responses and can transfer relatively large transgenes (>10 kb) compared to viral vectors which carry less than 10 kb, TEVs may facilitate clinical applications for gene therapy. A brief comparison between viral vectors and transposable element vectors is made in Table 1.

Transposons and induced pluripotent stem cells (iPSC)

iPSC are derived from autologous somatic cells after genetic reprogramming. iPSCs may have application in regenerative medicine. For the first time in 2006, Yamanaka, et al., created the first iPSCs from adult human fibroblasts by defined factors. Ectopic expression of a defined combination of four transcription factors (c-Myc, Klf4, Oct4, and Sox2) now known as the Yamanaka factors, originally noted to be overexpressed in embryonic stem cells by Yamanaka, can cause genetic reprogramming of mouse and human fibroblasts.[57,58] iPSCs are pluripotent like embryonic stem cells. Due to ethical concerns and histoincompatibility barriers, the use of embryonic stem cells is limited. iPSCs are derived from histocompatible, and autologous adult somatic cells, therefore they don't have these limitations. iPSCs can be genetically modified and similar to embryonic stem cells can differentiate into ectodermal, mesodermal and endodermal cells which can be used for degenerative and genetic disorders by transplantation (Figure 5).[57,58]

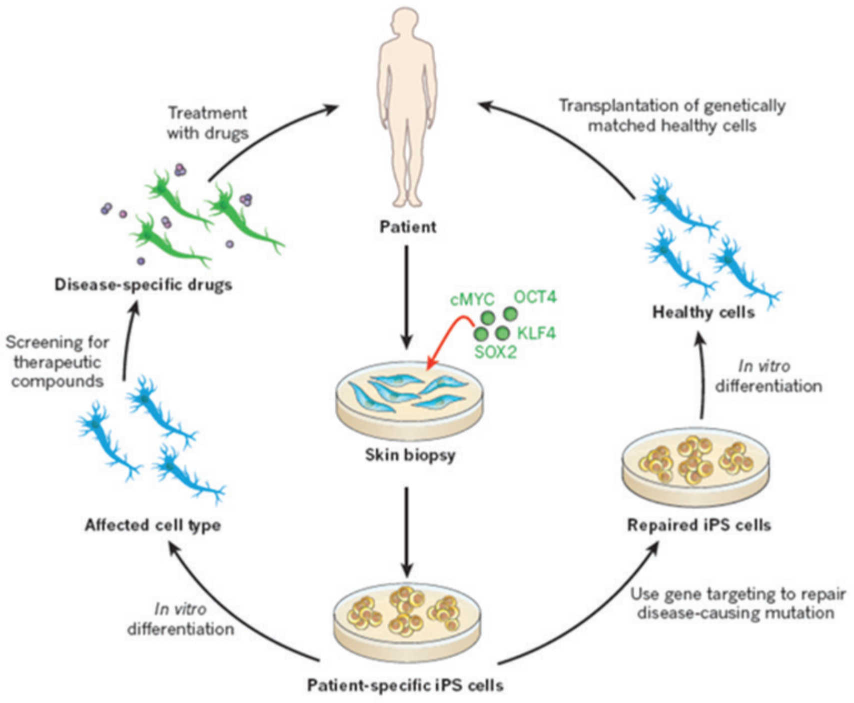


Figure 5. iPSC production and medical applications.[56]

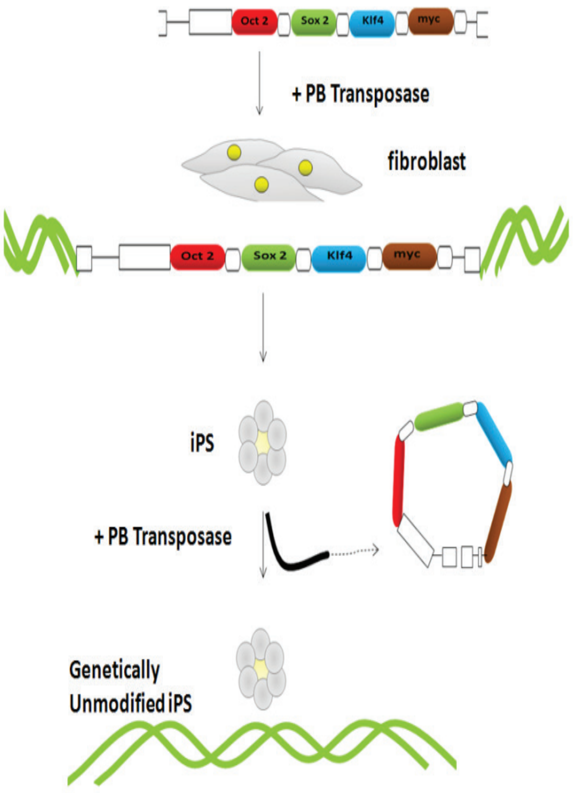


Figure 6. Piggybac's application in iPSC production.

Use of γ-retroviral and lentiviral vectors to insert the Yamanaka factors, essential for reprogramming, have some challenges[23]: the use of integrational viral vectors pose the risk of ectopic expression of the transgenes in the progeny of the reprogrammed cells. These two viral vectors have LTRs. Methylation of this region suppresses expression of transgenes and demethylation of LTR regions reactives the expression of these four factors in the iPSC progeny.[23] Klf4 and c-Myc are oncogenes and their reactivation in iPS derived cells can cause tumors as evidenced by Nakagawa, et al., studies in mice.[59] Insertional mutagenic ability of these two viral vectors may cause oncogenesis in the iPS derived progeny.[23]

Yamanaka's team has proven that integration of the Yamanka factors to generate pluripotent cells is not necessary. Repeated transient transfections with plasmids and proteins can generate iPSC.[61,61] Others have shown that viral vector-free integration of reprogramming genes followed by their removal is a more efficient and safer method.[62,63] These studies used a single *PB* vector to insert the four genes into the genome. Yamanaka factor genes were placed in one vector and each factor was separated from the other with a viral 2A oligopeptide (Figure 6) for post-translational cleavage of the polypeptide by synthesis of each factor from a single transcript. Transfection of the *PB* vector into mouse or human fibroblasts caused generation of bona fide iPSCs which differentiate into ectodermal, mesodermal and endodermal lineages. The efficiency of these iPSCs is consistent with results from γ-retroviral and lentiviral vectors which were evaluated by functional assays to prove pluripotent capability of the generating cells.[56] PB vectors have the ability to remove the exogenous gene from its insertion site without any footprint. For this reason, transient transfection of the *PB* transposase is necessary. This removal process of the exogenous gene and the transient expression of the *PB* vector can yield relatively robust reprogramming efficiency and bypass challenges faced with γ-retroviral and lentiviral vectors.[8]

Different groups have focused on perfecting iPSC generation techniques. Okita, et al., in 2008 used a multiprotein expression vector. [64] Kaji, et al., in 2009 worked on virus-free integration of genes followed by experiments to remove reprogramming genes.[62] For the first time in 2009, Woltjen, et al., and Kaji et al., used piggyBac vectors with high efficiency.[62,65] Subsequently, Okita, et al., in 2010 used plasmid vectors with multiple transient transfections. This study unfortunately showed low efficiency.[66] miRNAs are also being used to generate iPSCs. All of these studies show beyond doubt that transient expression of reprogramming factors in somatic cells is sufficient to reset their gene expression to the pluripotent state. Moreover, novel vectors have been designed containing the reprogramming genes followed by a poly-arginine tail which expresses reprogramming proteins with the ability to penetrate the cell and the nucleus.[67,68] The poly-arginine peptide enables the recombinant protein to readily enter the cell and have been shown to allow their translocation into the nucleus. This vector can be transfected into HEK293 mammalian cells for expression of reprogramming proteins and cell extracts containing expressed proteins can be used for reprogramming of target cells by simply adding the cell extract to the target culture media.[69] Another novel approach is by using Sendai virus (Se V) vector which is an RNA based vector without any possibility for integration into the host genome. Therefore, Se V could be a valuable tool for generating footprint-free iPSC with no genetic modification. In addition, blood samples and skin biopsies, both, have been used successfully to generate iPSCs but use of blood samples is minimally invasive in contrast with skin biopsies. Thus, generating safer iPSCs is possible.[70,71] In regenerative medicines, safe iPSCs is important and target cells can be reprogrammed without genetic modification.

Transposon based systems for gene therapy

The first successful gene therapy, in the year 2000, was accomplished by French scientists for X-linked severe combined immunodeficiency (SCID-X1) in children by using retroviral vectors. Unfortunately, two of the ten children treated with SCID gene therapy developed T-cell leukemia and died due to retroviral vector insertional mutagenesis through insertion and activation of the LMO-2 gene.[72] Until 2007, the most popular tools for gene therapy were viral vectors,[73] but viral vectors have genotoxicity and also provoke immune responses.[74] On the other hand, other gene delivery systems such as naked DNA and plasmids have low immunogenicity and low genotoxicity but have episomal feature translating into transient gene expression and low efficiency.[75] Now, the third generation of genetic vehicles, TBVs, has lower insertional mutagenesis than viral vectors and low immunogenicity features and can integrate larger than 10 kb GOI into the host genome with stable expression of GOI.[41,76,77] These characteristics plus transposition through the simple cut and paste mechanism make them powerful tools for gene therapy.

It is noteworthy that each TEV has its own features and can be distinguished. *SB* and *PB* vectors are the most common transposon based vectors that investigators have focused on for the past 20 years. Studies revealed that *SB*'s integration is almost random,

Table 1. Advantages and disadvantages of gene therapy vectors.

Vector type		Advantages	Disadvantages
Viral vector	AV	1) Transduce both dividing and non-dividing cells	1) Very high risk of immunogenicity 2) Limited size of insertion 3) Transient expression 4) Short time of existence
	RV	1) High level of expression because of robust promotors 2) Integrate to the host genome	1) High risk of genotoxicity 2) High risk of immunogenicity 3) Limited size of insertion 4) Methylation and silencing of GOI 5) Transduce only dividing cells
	AAV	1) High tropism 2) Never cause genotoxicity 3) Poor immunogenicity	1) Transient expression 2) Limited size of insertion 3) Short time of existence
	LV	1) Stable expression 2) Transduce both dividing and non-dividing cells	1) High risk of genotoxicity 2) High risk of immunogenicity 3) Limited size of insertion 4) Methylation and silencing of GOI
Transposon based vector	SBV	1) Integrate the GOI in to the genome 2) Low risk of genotoxicity 3) Hyper active transposition of *SB100X* transposase 4) Without immunogenicity	1) Transposase hard to manipulate genetically 2) Methylation and silencing of GOI 3) OPI seen in *SB*ase 4) Transposition causes a footprint
	PBV	1) Stable expression 2) Transposase easy to manipulate genetically 3) High transposition activity 4) OPI not seen. 5) Transposition without any footprint 6) Without immunogenicity	1) High risk of genotoxicity 2) Possibility to activate *PB* like elements
	Tol2	1) Stable expression 2) Carrying larger gene that two other transposon based vectors 3) OPI not seen. 4) Without immunogenicity	1) High risk of genotoxicity 2) Low transposition activity

without bias to any active genes, promoters or CpG islands. In contrast, *PB* and *Tol2* have the tendency to integrate into transcriptionally active genes, promotors and CpG island regions.[78,79] This means that it is more likely for *PB* and *Tol2* to cause insertional mutagenesis in the genome than *SB*'s. It is worth noting that the novel hyperactive *PB*ase has a different integration pattern with reduced tendency for integration near transcription start site.[44] In addition, since there are *PB* like elements in the human genome, introduction of *PB*ase may result in activation of *PB* like elements with undesirable consequences.[11] The same does not apply to *SB* which is evolutionarily far from the human genome. *SB*-like elements do not exist in the human genome and thus there is no justifiable fear for its activation.[80] Therefore, *SB* is safer for gene therapy.[80] On the other hand, *SB*ase is difficult to manipulate genetically for greater transposition efficiency. Instead, *PB*ase is more flexible and easily engineered for robust and targeted transposition activity (Table 1).[80] Generally, researchers currently prefer *SB* transposon/transposase constructs for gene therapy purposes and *PB* transposon/transposase constructs for generating iPSCs. It should be noted that there are new studies trying to combine the transposase enzyme with other site specific molecules like zinc finger proteins in both transposon based systems. The results of these studies are promising.[81,82]

In 2009, Xue, et al., used *SB* vectors with hyperactive *SB*ase (*SB*100X) and found stable gene expression in cord blood-derived $CD34^+$ hematopoietic stem and progenitor cells in NOG mice.[83] For the first time in the year 2000, Yant, et al., used transposon vectors as tools for gene therapy in liver of mice.[35] Since then, there have been many successful preclinical gene therapy studies benefiting from use of transposon vectors. The *SB* system has been used in delivering Factor VIII,[84–86] factor IX,[35,87] insulin,[88] and lysosomal enzyme.[89] In addition, the *SB* system utility has been studied for the treatment of epidermolysis bullosa,[90] tyrosinemia type I,[91] fanconi anemia type c,[92] Huntington's disease,[93] sickle cell anemia,[94] and mps I and VII.[89,95] Anti-cancer therapy via the *SB* system has been done in glioblastoma,[96,97] gastrointestinal cancer,[98] osteosarcoma,[99,100] and B-cell lymphoma.[101–103] Also, multifactorial diseases including diabetes,[88] pulmonary hypertension,[104] and jaundice[105] have utilized the *SB* system in experimenting with treatment strategies. The *PB* system has been used in a few gene therapy studies.[106–109] The first clinical trial applying the *SB* vector is being conducted in B lineage malignancies.[110] Table 2 is a non-comprehensive review of transposon tools used in gene therapy of various diseases.

From 2006 until now, four clinical trials using SBV for patients with B lineage malignancies are in progress at the MD Anderson Cancer Center (MDACC) (IND No.: 14193, 14577, 14739, and 15180).[120] In these ongoing clinical trials, engineered CD19-specific CAR$^+$ T cells have been developed using a binary system (*trans*) -SB transposon/SB11 transposase. This approach is a combination of immunotherapy with gene therapy techniques.[120,121] CAR$^+$ T cells were generated in several groups using viral vectors as a gold standard, which was time-consuming and costly.[120] In contrast, manufacture of SB plasmid vectors is cost efficient.[116]

Table 2. Transposon based Gene Therapy of Disease States.

Year	Disease	Vector	References
2000	Hemophilia	*SB*	Yant, et al.[35]
2002	Tyrosinemia type I	*SB*	Montini, et al.[91]
2003	Junctional epidermolysis bullosa	*SB*	Ortiz-Urda, et al.[90]
2004	Diabetes	*SB*	He, et al., 2004.[88]
2004	Glioblastoma	*SB*	Ohlfest, et al.[96]
2005	Huntington disease	*SB*	Chen, et al.[93]
2005	Glioblastoma	*SB*	Ohlfest, et al.[97]
2005	Hemophilia A	*SB*	Ohlfest, et al.[84]
2006	Hemophilia A	*SB*	Liu, et al.[85]
2006	Induced pulmonary hypertension	*SB*	Liu, et al.[104]
2007	Mucopolysaccharidoses	*SB*	Aronovich, et al.[89]
2007	Sickle cell disease	*SB*	Belcher, et al.[94]
2008	Lymphoid malignancy	*SB*	Singh, et al.[101]
2008	Cancer	*SB*	Huang, et al.[102]
2009	Cancer	*SB*	Xue, et al.[83]
2009	Mucopolysaccharidose I	*SB*	Aronovich, et al.[95]
2009	Hemophilia A	*SB*	Kren, et al.[86]
2009	Jaundice	*SB*	Wang, et al.[105]
2009	B-lineage malignancies	*PB*	Manuri, et al.[106]
2011	Fanconi anemia type C	*SB*	Hyland, et al.[111]
2011	T cell malignancy	*PB*	Nakazawa, et al.[107]
2012	osteosarcoma lung metastases	*SB*	Fujiwara, et al.[99]
2012	Hereditary Tyrosinaemia type 1	*SB*	Pan, et al.[112]
2012	Retinal degeneration	*SB*	Johnen, et al.[113]
2012	Liver disease	*PB*	Burnight, et al.[44]
2012	Modification of human primary T cell	*PB*	Saha, et al.[114]
2013	T cell lymphocyte malignancy	*PB*	Nakazawa, et al.[108]
2013	Hepatic gene deficiency disorders in mice	*PB*	Anderson, et al.[115]
2013	Sickle cell anemia	*SB*	Sjeklocha, et al.[116]
2013	T cell malignancy	*SB*	Huls, et al.[117]
2013	AML	*SB*	Tettamant, et al.[118]
2014	Pancreatic cancer in mice	*SB*	Park, et al.[119]

A chimeric antigen receptor (CAR) recognizes tumor associated antigen (TAA) and consists of multiple domains; scFv derived from TAA-specific mAb from mice, a flexible hinge (Fc), a transmembrane region and signaling endodomains.[122] Depending on the signaling motif, three generations of CAR currently exist; first generation contains only the activation signal ($CD3\zeta$), second generation contains the activation signal and CD28 costimulatory signal ($CD3\zeta$+ CD28), and the third generation has activation signal plus multiple costimulatory signals ($CD3\zeta$+ CD28+ OX40).[122,123] Cooper, et al., inserted the 2nd generation CAR gene in SB plasmid specified for CD19 (cell surface antigen in B cells). Then, the binary system was electroporated into autologous and allogeneic T cells, derived from cord blood cells. Engineered CAR$^+$ T cells were retrieved by co-culturing with artificial Antigen Presenting Cells (aAPC) expressing CD19 on their cell surface in presence of interleukin-2 and 21. Therefore, only CD19-specific CAR$^+$ T cells began to stably express CAR on their surface.[121,124] The advantage of CAR$^+$ T cells is that CAR recognizes TAA-CD19 independent of human leukocyte antigen (HLA).[120,121] Cooper, et al.'s protocols were published for manufacturing of clinical grade patient-derived or donor-derived CD19-specific T cells in 28 days after autologous or allogeneic hematopoietic stem-cell transplantation (HSCT), respectively.[120,124] Recently, attempts have been made to increase efficiency of transposition by replacing SB11 transposase with SB100X transposase[120] Despite these achievements, possibility of insertional mutagenesis using SBV as a gene delivery vehicle still exists.[125]

In conclusion, novel clinical gene therapy strategies will be achieved by development and improvement of transposon gene delivery systems. Currently, the most attractive systems for stable gene manipulation in primary somatic or stem cells are *PB* and *SB* transposon systems. The ability of *SB* transposon/transposase system for stable gene manipulation and expression is suggestive of future trends in gene delivery systems. There remain some challenges in these systems. For example, the genotoxic risks of *SB* and *PB* transposons must be analyzed in more detail. In addition, for better optimization and validation, safety insulator sequences could be used for site-specific targeted integration. It is important to experiment and test this system in all previously conducted experiments of viral vectors for comparison purposes.[126, 127] Aside the limitations and problems that current transposons have, it is necessary to plan studies in animal models and preclinical studies to prepare for future gene therapy clinical trials like the ongoing clinical trials using SBV in B lineage malignancies at the MD Anderson Cancer Center.

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