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RESEARCH ARTICLE

DNA TRANSPOSONS (NON - VIRAL VECTOR) FOR GENE THERAPY

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ABSTRACT

Gene therapy offers important perspectives in current and future medicine but suffers from imperfect vectors for the delivery of the therapeutic gene. Most preclinical and clinical trials have been based on the use of viral vectors, which have evident advantages but also some serious disadvantages. In the past decade the use of DNA transposon-based systems for gene delivery has emerged as a non-viral alternative. DNA transposon vector engineering remains largely in a preclinical phase but some interesting results have been obtained. This mini-review aims to provide the current state of the art on DNA transposon vectors used in a gene therapy perspective.

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INTRODUCTION

Gene therapy has emerged as a promising method to cure genetic diseases or at least improve the status of patients. If the idea of replacing an "abnormal" dysfunctional gene by its "normal" functional counterpart may look simple, technical difficulties are often encountered such as the stable and targeted insertion of the therapeutic gene into the host genome. Several gene therapy strategies can be distinguished that address different kinds of pathologies. Recessive monogenic hereditary diseases, such as cystic fibrosis and Duchenne's muscular dystrophy, could be treated by the addition of the "normal" gene counterpart that will produce a sufficient amount of functional protein to reverse the disease. However, some other monogenic defects will require the replacement of the "abnormal" gene by its "normal" allele and not solely its ad- dition. In some cases of acquired and/or polygenic diseases such as diabetes, the replacement of one of the deleterious alleles or the addition of one "normal" gene may be sufficient to fight the disease. Moreover, in complex pathologies that mix genetic predisposition with environmental factors (cancer diseases for example), different gene therapy strategies may be useful, such as the extinction of a targeted gene expression or the introduction of a suicide gene. The common challenge is to introduce the new DNA durably into the genome of pathologic cells without disturbing the regulation of "normal" gene expression. This is the main objective of vector development. Over 1340 human gene therapy clinical trials were completed in 28 countries between 1989 and 2007 (1). However, very few phase-II and phase-III clinical trials have been conducted that mainly use viruses as vectors (2). Following a brief description of virus-based vectors, the advantages offered by DNA transposons for gene therapy will be presented with some examples of successful applications

Vectors for gene therapy (Viral and non-viral) : Viruses, liposomes, and naked DNA are some of the vehicles used to introduce transgene into the host genome. The vehicles used to introduce the transgene is known as vectors, the utility of the vector depends on the factor enlisted below,

- The size of the exogenous gene (transgene)
- The efficiency of the delivery
- It will induce the host immune response or not
- The stability and longevity of the transgene
- Level of expression of a transgene

"If the selected vector can carry larger transgene that cannot induce immune response with good efficiency to infect the cell and higher expression rate, are selected for the gene therapy experiment." The classification the gene therapy vectors are given into the figure below,

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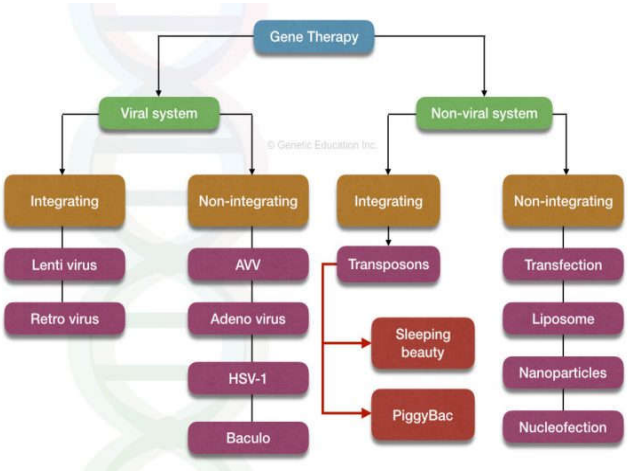
The vectors are divided into two broader categories

- Viral vectors
- Non-viral vectors



Virus-based vectors: promise and disappointment: Virus-based vectors are the most commonly used gene delivery system because of their ability to integrate the therapeutic gene into the host genome and ensure stable and long term gene expression. The second advantage of virus-based vectors is the combination of the DNA vector with a highly efficient DNA delivery system in the cell, *i.e.* the natural infection process. The main virus- based vectors used are derived from retroviruses (especially lentiviruses), adenoviruses and adeno-associated viruses (2,3). For example, 68% of the gene therapy clinical trials completed in to date used virus-based vectors (1,2). Unfortunately some fatal adverse events have clouded the sky. In 1999, a patient treated for ornithine transcarbamylase deficiency with an adenoviral gene transfer developed a fatal systemic inflammatory response syndrome (4). In 2000, a French clinical trial using a retrovirus-based gene therapy on children suffering from X-linked severe combined immunodeficiency syndrome (X-SCID) seemed more promising, and 10 patients appeared successfully cured (5). However, two years later two of the ten children treated developed T-cell lymphoma and one died (6). This major adverse event wsystem in the cell, *i.e.* the natural infection process.

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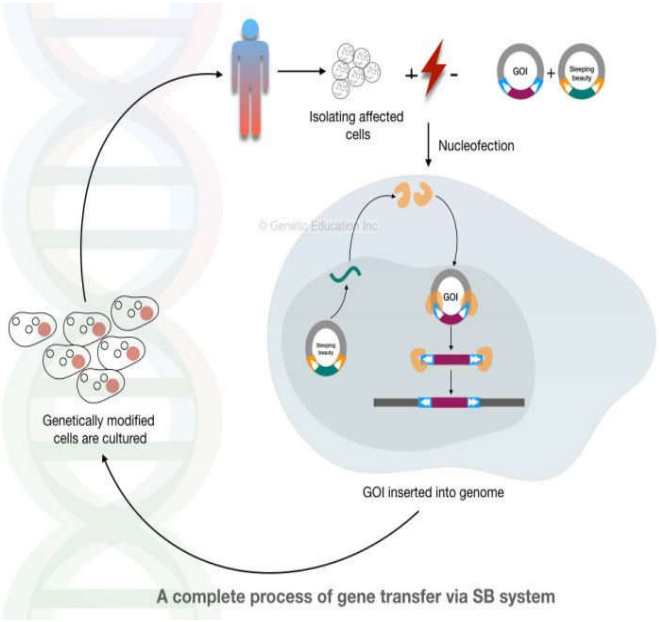
Non-viral vector: Liposome, naked DNA, nucleofection, and transposons are some of the non-viral vector-mediated methods used for the gene therapy. Why the non-viral vector will be one of the best opportunities for the gene transfer? The non-viral vectors are non-toxic, non-immunogenic, and tissue-specific. Also, it is easy to design and apply them. Some of the non-viral vectors are discussed here below.

Liposome: The liposome also called lipoplex-mediated gene therapy is an artificial technique non-infected to the host cell. The liposomes are artificially synthesized molecule typically 0.025 to 2 µm in size, made up of the lipids. The DNA cannot directly be inserted in the cell because the cell surface, as well as the DNA itself, has a negative charge. Hence naturally both repel each other. The liposome is used to solve this problem. The lipid molecule is hydrophilic as well as hydrophobic in nature which protects the interior aqueous phase. The head of it is hydrophilic while the tail is hydrophobic. The liquid solution of DNA is encapsulated with the liposome lipid bilayer which helps in penetrating it. Nonetheless, due to the smaller size of it, the liposome is not a good choice for the larger size of gene transfer. Also, the liposome is non-attractive for the DNA as well as the cell surface. This problem is encountered by introducing a positive charge on the hydrophilic domain of the lipid. A new type of upgraded version of the liposome is designed and named it as lipoplex. The lipoplex attracts both the DNA as well as the cell surface, hence, a more stable complex of lipid-DNA is created. This tube-like structure efficiently transfers DNA into the target

cell. Plus, lipoplex is non-immunogenic. Due to this reason, it is the best choice over the liposome and virus-based vector. Furthermore, it is easy to prepare and can transfer a large amount of DNA.The major disadvantage of the lipoplex is that it is not as efficient as the virus-based gene therapy.

Transposon: Transposon-Mediated gene therapy is one of the emerging therapy after the CRISPR-CAS9. Why transposon? The transposons are mobile genetic elements that can move from one location to another into the genome.It also contains coding genes and terminal repeats as like the viruses. However, almost all transposons are inactive for long.The scientist has discovered active transposons from the fish fossil and named it as sleeping beauty transposon. The SB system is capable of inserting DNA into the host genome without any side effects. It escapes the host immune system too and delivers a gene of interest efficiently at the target site. Although the method still has many loopholes and limitations that need to be perfected before any pre-clinical trial. The general mechanism of the SB system is shown in the figure below.



Nevertheless, systems like SB transposon and CRISPR-CAS9 will become more effective in the future. We had explained sleeping beauty transposon in our previous article, the article contains all the information on the SB system, starting from their discovery to its mechanism of action.

Dna transposon-based vectors: DNA transposons are a group of transposable elements present in most (if not all) organisms from bacteria to mammals (20), but poorly represented in the human genome (21). The main families of DNA transposons (*Tc*1/ *mariner*, *hAT* and *PiggyBac*) have a simple structure with a unique gene flanked by inverted terminal repeats (Figure 1(a)). Their mobility is supported by the transposon-encoded transposase enzyme in a "cut and paste" mechanism.

(Figure 1(b)). This simple genome integration mechanism makes DNA transposons very attractive as gene delivery tools. To achieve this goal a two-plasmid system should be developed consisting of a helper plasmid expressing the transposase and a donor plasmid with the terminal repeat sequence flanking the gene of interest (Figure 2) (22-24).

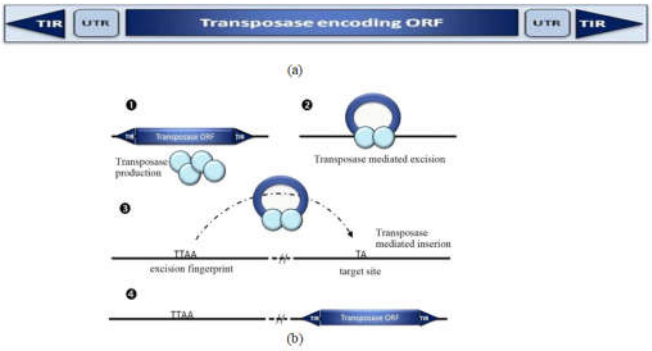


Figure 1. (a) Structure and (b) simplified "cut and paste" transposition mechanism of a DNA transposon. ORF, open reading frame; TIR, terminal inverted repeats; UTR, untranslated region. The light blue circles in B represent the transposase proteins encoded by the transposon ORF (b1). Transposase dimer (or more) binds to ITR (b2) and excises transposon DNA, then the transpososome is displaced (b3) to be integrated in another genomic site (b4).

Using this system, several DNA transposons, such as *P* element, *PiggyBac*, *Tol*2, *Hsmar*1 and *Sleeping Beauty*, have been utilized for gene delivery and mutagenesis in invertebrate and vertebrate cells (24- 33). Among the DNA transposons, *PiggyBac*, *Tol*2 and *Sleeping Beauty* have been evaluated for gene therapy in animal experiments, primary cell gene delivery and a few pre-clinical trials (34-37). The advantages and disadvantages of DNA transposon-based gene delivery will be discussed below with respect to cargo capacity, efficiency, gene silencing and targeted insertion with special emphasis on *PiggyBac*, *Tol*2 and *Sleeping Beauty*.

Cargo Capacity: A large cargo capacity is desired for therapeutic application and DNA transposon-based vectors appear as good candidates with respect to this criterion. Indeed *Tol*2 and *PiggyBac* vectors are able to integrate up to 11 and 9.1 kb of foreign DNA respectively (28,38). Furthermore the large cargo capacity of *Tol*2 was further evidenced with a ~70 kb BAC transfer in mouse and zebrafish genomes (39). By contrast the transposition activity of wild type *Sleeping Beauty* is significantly decreased when foreign DNA exceeds the initial 1.7 kb size (40).

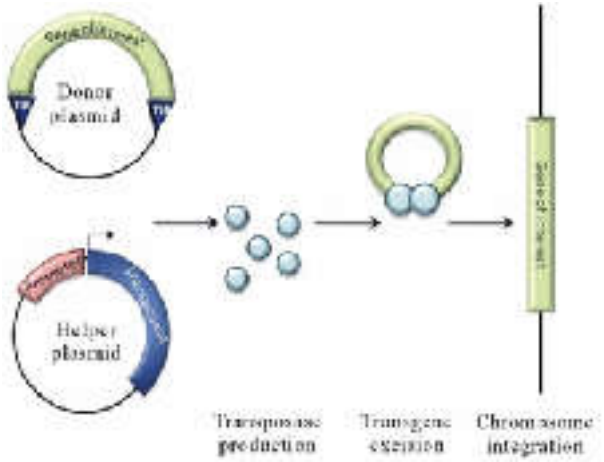


Figure 2. Schematic view of a DNA-transposon system for gene delivery. Both donor and helper plasmids are transfected into the host cells. The helper plasmid is an expression vector that allows transposase production and the donor plasmid bears the gene of interest that will be integrated into the host genome by the transposase. The light blue circles represent the transposase proteins produced from helper plasmid.

Transposition Efficiency: The transposition efficiency of *PiggyBac*, *Tol*2 and a mu- tant *Sleeping Beauty* transposase (SB11) was compared in several human cell lines. *Tol*2 and *PiggyBac* were highly active in all cell lines tested while SB11 was obviously less efficient and marginally or totally inactive in two cell lines, *i.e.* HeLa and H1299, respectively (30). However, a new hyperactive *Sleeping Beauty* transposase (SB100X) displays transposition efficiency comparable to *PiggyBac* under non-restrictive conditions (41). So, transposase mutagenesis may be an interesting way to increase DNA transposon-based vector efficiency as shown for *Sleeping Beauty* hyperactive mutants (41,42) and hyperactive or mouse-codon optimized *PiggyBac* (43,44).

Gene Silencing and Stability of Gene Insertion: Any transgene introduced into the host genome is a potential target for the position effect that causes transgene silencing. This phenomenon is observed particularly in viral vectors but also appears in plasmid and DNA-transposon vectors. However, in the study by Grabundzija *et al.* transgene silencing observed following DNA-based gene delivery using *Tol*2, *PiggyBac* or SB100X, was found to be very low (3.8% vs 26.5% in control) (45). This result has to be moderated since earlier results reported progressive post-integrative gene silencing after *Sleeping Beauty* transposition (46). Furthermore post-integration transgene silencing may be higher than pre- viously observed as suggested by Meir and Wu (47) indicating 63% for *Sleeping Beauty*, 46% for *Tol*2 and 20% for *PiggyBac*. A comparative study between viral vectors and non viral vectors used under the same conditions may be of interest to decide whether DNA transposon-based vectors have advantages or not with respect to the gene silencing effect (47). Interestingly, reducing the terminal repeats of *PiggyBac* to 40 bp and 67 bp from 245 bp and 311 bp of 3' and 5' terminal repeats respectively in the commonly used *PiggyBac*, enhances *Piggy Bac*'s transposition activity (48). This suggests a possible interaction between epigenetic silencing factors and sequences present in the common *PiggyBac* but not in its shorter form. Furthermore, this shorter *PiggyBac*, called micro-*PiggyBac*, lacks many activator sequences initially present in the 3' *PiggyBac* terminal repeats that influence neighboring gene expression (49). In addition, transgene delivery without drug selection is advantageous for therapeutic purposes but may interfere with stable insertion. A recent study compared the stable insertion ability of the *Tol*2, *PiggyBac* and *Sleeping Beauty* DNA-transposon gene delivery systems in human peripheral blood and umbilical cordblood-derived T-cells (50). This study showed that *PiggyBac* is the most efficient of the three DNA transposon systems in mediating stable gene insertion.

Insertion Site Targeting: Site targeting is a crucial point in gene transfer and especially in the human gene therapy perspective. Most of the integrating viral vectors have integration site preferences leading to an integration site bias that may explain some adverse effects such as gene silencing or activation of cancerrelated genes (51-54). By contrast, genome-wide targeting profiles of *PiggyBac*, *Sleeping Beauty* and *Tol*2 in various human cell lines and human primary cells clearly demonstrate that the three transposon systems display a lower frequency in targeting near cancer-related genes and less bias in targeting active genes compared to viral-based vectors (29,45,48,50,55, 56). A detailed analysis of this aspect is available in Meir and Wu (2011) (47). The ability of *PiggyBac* transposase to be molecularly engineered without a loss of activity allows the construction of *PiggyBac* transposase chimeras with a DNA binding domain, such as the zinc finger DNA binding domain that would recognize specific and safe integration sites.

Drawbacks and future challenges: The DNA transposon-based gene delivery systems encounter some drawbacks which must be overcome, some being shared with other viral and non-viral vectors. The behavior of DNA transposon in different cell types remains largely unknown, especially regarding *PiggyBac* and *Tol*2. One might worry some cytotoxicity of transposase overproduction, leading to overproduction inhibition. Studies with *Sleeping Beauty* have been conducted in numerous cell types showing a cell type dependent activity and the interaction with human factors such as HMGB1 and Miz-1 (24,36,45). Unlike *Sleeping Beauty*, *PiggyBac* transposition seems to be cell type independent. However cytotoxic interaction of *PiggyBac* with cellular factors cannot be ruled out since a little overproduction inhibition could be observed (30,44,47).

The existence of *PiggyBac* like elements in the human genome (21) raises the question of safety concerns. Indeed a transmobilization of endogenous elements by the engineered transposase might be mutagenic. This does not apply to Tc-1 like elements such as *Sleeping Beauty*, which have no close relatives in the human genome. This is also one interest to develop new vectors based on distant sequences (*e.g.* from crabs···) (22,). However the longer the foreign sequences introduced into the host genome, the greater the probability of evoking adverse consequences such as gene silencing and dysregulation of genes nearby. Therapeutic gene silencing and dysregulation of genes nearby are common drawback of DNA transposon-based vectors and viral vectors (3,47). Epigenetic gene silencing was observed post-integration with *Sleeping Beauty* that was influenced by DNA methylation and histone deacetylation (46). Epigenetic regulation of *PiggyBac* vector was also suggested by the results of Meir *et al.* (48).

Another concern shared by both non-viral and viral vectors, is the integration site specificity and its counterpart insertional mutagenesis. To overcome this point is the major challenge for gene therapy, and despite years of research, this has not been addressed to date. The addition of specific DNA binding domain, such as zinc-finger domain of known transcription factors, appears inefficient and reduces the transposition rate. Some outlook may come from artificial zinc-fingers which harbor modular structure and function making possible to recognize theoretically any sequence in the genome (37).

Finally, to my point of view, the major concern of DNA transposon-based delivery systems is to keep open the problem of DNA delivery into cells, with the drawbacks of all non-viral delivery systems (mainly toxicity and low efficiency). It is beyond the scope of this review to address the pros and cons of non-viral delivery systems (e.g. liposomes, polymers, electroporation···) but it is a great challenge to develop efficient and non-cytotoxic re- agents for DNA delivery across the cell membrane. May- be the future resides in the hybrid technology combining the natural ability of viruses to cross cell membranes with efficient genomic insertion of DNA-transposon. Several *Sleeping Beauty*-virus combinations have been tried with herpes virus or lentivirus in human cells and adenovirus in mouse liver.

Conclusion

Because of their lower immunogenicity and the fact that they are less prone to gene silencing than viral vectors, DNA transposon-based vectors appears to be good and more desirable therapeutic gene delivery system. *Piggy Bac* has some advantages over *Sleeping Beauty* or *Tol2* because *Piggy Bac* transposase can be molecularly modified without substantially losing its activity (47). According to Meir and Wu, 2011, *Piggy Bac* seems currently the most promising DNA transposon to obtain a highly efficient gene delivery system capable of targeting the therapeutic gene at a predefined safe location in the host genome where the transgene can be stably and faithfully expressed without disturbing global gene expression. However, this ambitious goal has not yet been achieved and much progress is still required. Finally, improvement of cargo capacity, delivery efficiency and expression stability/persistence may be achieved with chimeric vectors combining viral (*i.e.* baculovirus or adenovirus) and non viral vector (*i.e.* DNA transposon) elements such as those recently developed.

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