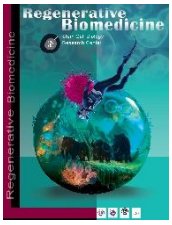

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Mini-Review Article

Lentiviral Delivery System: A Revolutionary Approach in Gene Therapy

Fatemeh Eslamizadeh[1], Ehsan Farashahi-Yazd[1*], Seyed Mahmoud Arab Najafi[2]

1. Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Cell and Molecular Biology Department, School of Biology, College of Sciences, University of Tehran, Tehran, Iran.

***Corresponding Author:**
Farashahi-Yazd, Ehsan
Email:
ehsanfarashahi@ssu.ac.ir

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Abstract

In the early 1970s, gene therapy was utilized for the first time to modify the genome or biological characteristics of living cells. Viral vectors have emerged as a promising method for transferring genes in therapeutic applications, due to their ability to infect a host cell and replicate. Adenoviruses, adeno-associated viruses, gamma-retroviruses, and lentiviruses are among the significant virus classes that have been studied in research trials so far and the first lentiviral gene delivery system was created by Naldini et al. in 1996.

However, despite the promising results, long-term patient monitoring is crucial to ensure the safety and efficacy of the vectors used over an extended period. This monitoring is necessary because viral vectors can have unintended consequences, such as activating oncogenes or causing immune reactions. Therefore, researchers and clinicians must continue to gather data and insights into the long-term effects of lentiviral vectors to ensure their safe and effective use in gene therapy.

Keywords: lentiviruses, viral vectors, gene transfer, lentivector generations, clinical applications.

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Introduction

For the first time in the early 70s, gene therapy was used to make alterations in the genome or the biological characteristics of living cells. Viral vectors have appeared as promising vehicles for transferring genes in therapeutic approaches, which is due to their ability to infect a host cell and replicate. So far, significant classes of viruses have been examined in research trials, including adenoviruses, adeno-associated viruses, gamma-retroviruses, and lentiviruses.

Review of related literature

The first gene therapy trial ever used Shope papillomavirus for three patients of hyperargininemia. The disease was treated by virus-encoded arginase. Nevertheless, the correction faced failure in the patients with no reduction of the arginine level in their blood (1). Many efforts were made to better understand the mechanism of reverse transcription and infection in RNA viruses and to improve the transformation of viruses into viral vectors used for therapeutic gene transfer.

The first successful gene therapy was carried out in 1991, correcting severe combined immunodeficiency through transducing the patient's peripheral blood CD34+ cells by γ-retrovirally encoded adenosine deaminase (2).

In 1996, Naldini et al. (3) reported the first lentiviral gene delivery system generation. The group used a type of production system called 'split-component' to divide the lentivirus genome into some plasmids. As each component is made less and less complete, viral production becomes possible only in packaging cells, but not beyond it. Based on this issue, different generations of lentiviral gene delivery systems were developed. In the first generation, although most of the HIV-1 genome is conserved, using the envelope gene of another virus raises the tropism of the vector. The most widespread envelope glycoprotein is taken from vesicular stomatitis virus, allowing the vector to infect a wider range of cell types. To ameliorate the safety of these systems in the second and third generations, some elements were excluded and/or rearranged (Figure 1) (4). The use of those generations is context-dependent, and the gene therapy efficacy depends on the quality of lentiviral vector transduction.

To date, the earliest successful HIV-1-based lentiviral delivery system, VRX496, has been effective in treating 65 cases in several trials. In the first phase, autologous CD4+ cells from HIV-1-infected subjects were modified by VRX496 ex vivo, containing an antisense sequence to the HIV-1 envelope gene, and then the cells were reintroduced into the body (5). As a result, destroying HIV RNA led to a reduction in the HIV replication and a rise in the CD4+ cell count. Since then, a large number of studies have been conducted to scrutinize these systems and boost their efficiencies. In 2017, the first lentiviral gene therapy-based product, known as Kymriah™, was approved by the FDA to serve as a CAR-T cell therapy in pediatric and young adult patients with ALL. So far, this system has been used in more than 250 clinical studies (6).

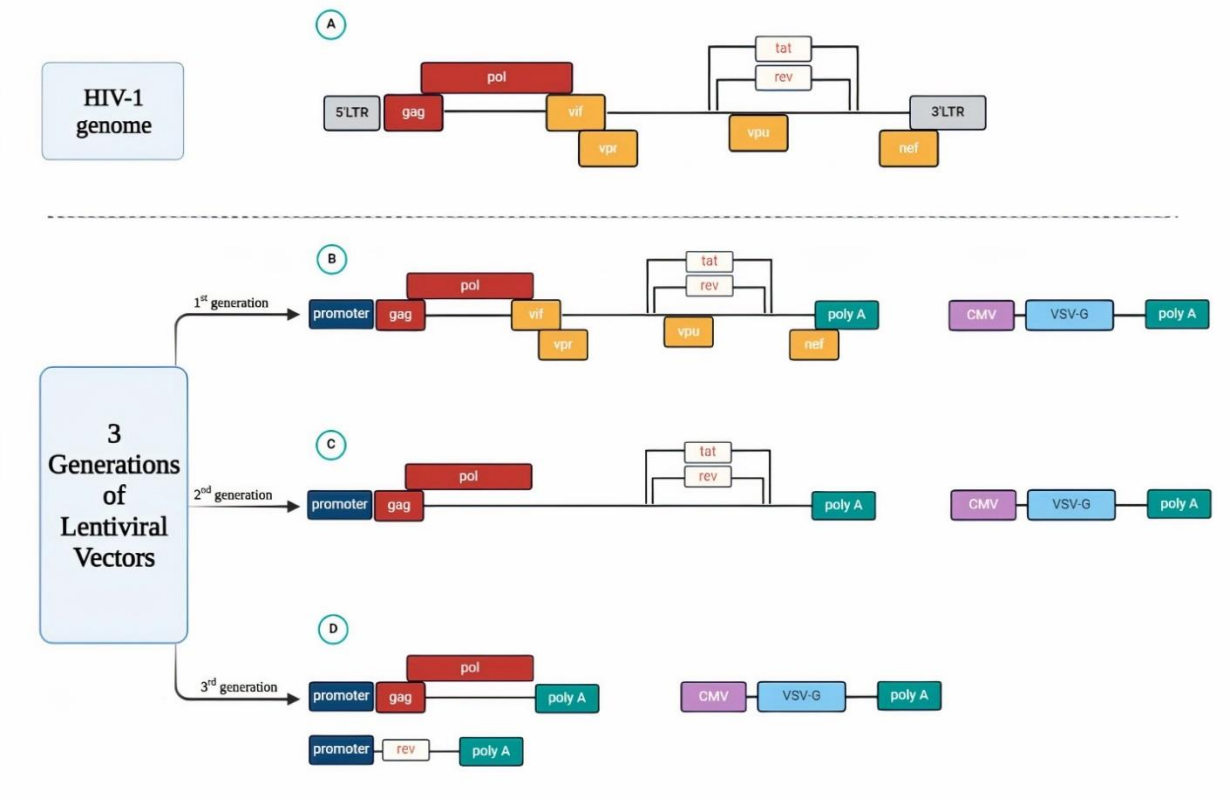

Figure 1. The schematic of the wild HIV-1 genome (A) and three generations of lentiviral delivery system (B-D). (B) In the first generation, all of the structural genes (gag, pol, and env-VSV), regulatory genes (rev and tat), and accessory genes (vif, vpr, vpu, and nef) are presented in the vector structure. (C) The accessory proteins-coding gene is not expressed in the second-generation lentiviral vectors. (D) rev-coding gene is modified in a separated vector, following tat removal in the third generation. LTR (long-terminal repeats); CMV (cytomegalovirus); VSV-G (vesicular stomatitis virus G); gag (group-specific antigen); pol (DNA polymerase); env (viral envelope); rev (transactivating protein); tat (trans-activator of transcription); vif (viral infectivity factor), vpr (viral protein R); vpu (viral protein u); nef (negative regulatory factor).

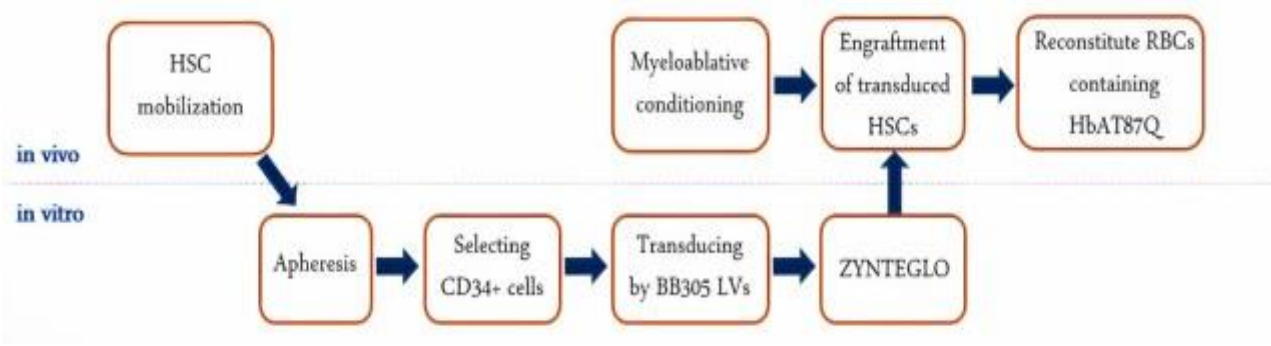

Figure 2. The procedure of ZYNTEGLO production in vivo and in vitro.

Gradually, some refinements were made to improve the safety and performance of the systems, such as 1) producing self-inactivating vectors to overcome replication-competent lentiviruses and 2) making integrase-deficient vectors to deal with the insertional mutagenesis issue. Moreover, utilizing tissue-specific promoters and miRNAs ended in targeted gene therapies using lentiviral systems (4).

There are several benefits to gene therapy using lentiviral systems. This approach relies on a genome-integrated gene delivery, so it provides a long expression of the target gene. One of the best-known products in the lentiviral delivery system is ZYNTEGLO, a population of autologous CD34+ cells containing patients' hematopoietic stem cells. These cells are modified ex vivo by the BB305 lentiviral vector-encoded β-globin gene to treat patients suffering from β-thalassemia. The procedures of HSC mobilization and cell harvesting should be undertaken individually for each patient. After CD34+ selection and ZYNTEGLO production, the cells are transplanted into the bone marrow to regenerate hematopoietic cells. This results in the production of normal Hb (Figure 2) (7).

Another primary advantage of lentiviral systems is their high payload capacity. In a study conducted on cells with Duchenne Muscular Dystrophy, a full-length dystrophin with a length of more than 15kb was delivered. The success of the process was then demonstrated by comparing the PCR product of transgene and the native coding sequence of dystrophin (8).

With this system, in vivo gene transfer into non-proliferating cell lines would occur as the proliferation of cell lines, like hepatocytes and neurons. This was demonstrated in Naldini and coworkers' report in 1996. Afterwards, gene transferring into rat neurons using the first generation of the lentiviral system was confirmed by immunohistochemistry tests (9).

Conclusion

Until now, the immense potential of viral vectors has been shown to deliver a gene of interest to diverse targets. There are several extensive lentiviral vector-based cell therapies in late-stage clinical development. Despite this, long-term monitoring of patients who have undergone the therapy is still required to gain knowledge of how to achieve the prolonged safety and success of the vectors used.

Conflicts of interest

The authors confirm that there are no conflicts of interest.

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