Mini Review

iMedPub Journals
www.imedpub.com

Cellular & Molecular Medicine: Open Access

2021
Vol. 7 No. 2: 10

Gene Therapy: A Detailed Review

Muhammad Rizwan Hamid*, Muhammad Sajid* , Muhammad Ahsan Shahzad, Muhammad Awais, Muhammad Sajid, Muhammad Aoun Haider, Asad Nawaz

Department of Biotechnology, Faculty of Life Sciences, University Of Okara, Okara, Punjab, Pakistan.

***Corresponding author:** Muhammad RH

ch.m.awais381@gmail.com

Department of Biotechnology, Faculty of Life Sciences, University Of Okara, Okara, Punjab, Pakistan

Citation: Hamid MR, Sajid M, Shahzad MA, Awais M, Sajid M, et al. (2021) Gene Therapy: A Detailed Review. Cell Mol Med. Vol. 7 No. 2: 10.

Abstract

For the treatment of a disease, transfer hereditary material into a patient is recognized as gene therapy. Gene therapy is known for introducing viruses into shuttle vectors which provide desired genes to the cells. For this purpose, various vectors such as viral or non-viral have been used. Two main methods i.e. *ex-vivo* or *in-vivo* have been used. Adeno, retro, and adenoassociated viruses have been used for gene therapy. Viral vectors are efficient over non-viral, but non-viral have some advantages over viral such as a better capacity for containing more DNA and very less immunogenicity. For enhancing the ability of non-viral vectors artificial viruses have been developed by inserting the receptors for better uptake and DNA translocation. Gene therapy for humans for the treatment of different diseases such as cancer acquired and genetic diseases already have been approved. Different methods have been developed for the uptake of DNA or transferring genes which provide better efficiency.

Keywords: Hereditary; Gene therapy; Immunogenicity; Vector

Received: February 28, 2021, **Accepted:** March 21, 2021, **Published:** March 29, 2021

Introduction

Many scientists work on gene therapy for the treatment of genetic diseases and allowed for treatment of the disease of single-gene and also used for various medicines. In the treatment of gene therapy, nonfunctional genes are replaced by healthy genes. Genes are units containing information for synthesizing proteins, proteins perform all activities of the body and also make the most weight of the cell. Particular protein performs a specific function but when genes changed or amino acid inserted wrongly in protein then genetic diseases occur and protein cannot perform normally [1]. In immunodeficiency disease, gene therapy is very effective and successful and showed long-lasting effects. mRNA must have transferred in cell and must be expressed insufficient amount. Rosenberg with his colleague did the first gene therapy by inserting neomycin gene marker through viral vector into lymphocytes of metastatic melanoma patients. In 2003 China approved 1st drug for gene therapy of malignant tumors. Now a day, in laboratories gene therapy is a significant area, and now is experimental application. It is used for the treatment of genetic disorders, cancer, and viral infection like AIDS and its trial have been conducted in the USA, Australia, and Europe. The main technique that is used for this purpose is recombinant DNA technology which is used to transfer the gene into a vector and then into the cells [2].

History of Gene Therapy

In recent years, the reputation of gene therapy was not fruitful. In between the 1970s-1980s two unofficial trials were started. Initially, in a trial, two young age girls suffering from arginase deficiency syndrome treat within *in vivo* method of gene therapy by using wild-type papillomavirus hoping that arginase may replace by viral arginase in patients [3]. In the second attempt for treatment of β-thalassemia in two patients, the *ex-vivo* method of gene therapy was used in which plasmids that were containing β-globin treated with the bone marrow, and bone marrow transplanted. Cases were not followed but there was no result (it was not harmful neither good) and all these trials were stopped [4]. In the 1990s officially two *ex-vivo* trials of gene therapy were started in the USA. In 1st one for treatment of Severe Combined Immunodeficiency (SCID) an enzyme was inserted into the T-cells. And in 2nd trial for the treatment of melanoma, cytokine extracted from the tumors was inserted in the lymphocytes and retroviruses have been used for cell transfection in both experiments all trials were failed [5]. From the 90s to onward trials were rapidly increased and RAC/NIH approved about 84 trials in just 1999. From 1999 to 2000, in 500 gene therapy trials about 4000 patients were treated. Protocols about 77% were

held in the USA and 69% of treated patients were cancer diseased and just 1% of protocols reached stage three. In 1999 at the University of Pennsylvania an 18 years' patient died during gene therapy treatment of liver disease, with that incident 90s decade ended very badly for gene therapy. In 2005 European Evaluation of Medicinal products received an application for drug approval of brain tumor treatment. Despite having the worst decade gene therapy is now have been confirmed. For the last five years for the treatment of HIV and AIDS therapies successfully have been done [6].

Approaches for gene therapy

For replacing faulty genes, a proper functioning gene introduced anywhere in the genome this method is used most commonly [7]

By homologous recombination method, faulty gene swapped for a proper functioning gene

By reverse mutation method repair the faulty gene which can perform normal functions again

Regulation of faulty gene can be changed

Gene Therapy Methods

Target genes can be transferred *in vivo* with inoculation or through *ex-vivo* in which outside the body targeted gene-modified and then introduced into the body again. Modified genes or DNA can be inserted into the organism through transfection or transduction [8].These techniques of transfection may involve, increase the permeability of the membrane, endocytosis, direct DNA injections, or receptor-mediated uptake of DNA. The transformation of genes relies on viruses in the Transduction method. Transferring of modified DNA exploited through techniques of Physical, chemical, and electrical. Transferring modified DNA through non-viral methods can decrease the risks of viruses such as mutagenesis and immune responses [9,10].

- Physical Method
- DNA injection directly
- Liposome moderate DNA insertion
- Transfection of calcium phosphate

Electroporation

Vectors of retroviruses

Other different viral vectors

Insertion of genes through receptors

Artificially synthesized chromosomes

Activation of functionally closely related genes

Site-directed recombination

Physical techniques for delivery enhancement

Electroporation: For transferring the DNA into the cells across the membrane, by providing high voltage pulses to the cells which can create pores temporarily in the membrane and then uptake the foreign DNA. Clinical use of electroporation is limited because its ranging towards cell types is efficient but it can cause cell deaths that's why it has been limited [11].

Gene Gun: Gene gun is a physical technique in which DNA transfer to the cells through particle bombardment, after coating the DNA with gold particles, this sample loads into the gene gun which bombarded the particles with pressure onto the cells and penetrated in them [12]. If in genome DNA penetrated in the wrong target it can create problems for patients, for example, it can produce tumors in suppressor genes of tumors. This problem already has been faced in trials of treatment of X-linked severe combined immunodeficiency disease in which through retroviruses hematopoietic cells were transferred in 20 patients and later in 3 of them have been developed T-cell leukemia [13].

Sonoporation: For transferring the DNA into the cells ultrasonic frequencies have been used in which the membrane of the cell is disrupted and DNA moves across the membrane known as the acoustic cavitation process.

Magnetofection: In this physical technique DNA is complexed with magnets, dishes of tissue cultures keep on the magnets and bring complexes of the DNA in contact with a monolayer of the cell this process is usually known as magnetoreception [14].

Chemical Techniques for delivery enhancement

Oligonucleotides: Using gene therapy treatment of various diseases artificially synthesized oligonucleotides used for inactivating faulty genes [15]. However, there are multiple methods to achieve this target but one of them is to stop transcription of the faulty gene by using antisense strands. In another, by stopping transcription of diseased involving genes through cleaving mRNA strands by using siRNA molecules.

Lipoplexes and polyplexes: For transferring DNA into the cells and improving its delivery it should be prevented from damages, for vectors synthesizing initially lipoplexes developed by using anionic and lipids [1-4].

Sonoporation: For transferring the DNA into the cells ultrasonic frequencies have been used in which the membrane of the cell is disrupted and DNA moves across the membrane known as the acoustic cavitation process.

Dendrimers: These are macromolecules and form branches and having spherical shapes. The surface of these molecules can be used for various functions and due to their surface also various properties of functions can be analyzed. Dendrimers can be developed with positive charge and DNA or RNA present there can make associations temporarily with dendrimers and then through endocytosis this DNA complex uptake by the cells.

Hybrid methods: Shortcomings resulting almost in every gene inserting method so that's why hybrid methods also have been developed by joining two or more techniques. For example, Virosomes are a hybrid which is a combination of HIV or influenza virus with liposomes. This hybrid method provided better-transferring efficiency than viral and liposomal methods [12].

An electrical technique for delivery enhancement

For gene transferring to the cells electro-transfer is a more efficient method, by applying electrical field permeability of the cells can be increased and though structural changes (electro-pores) occur in the cell membrane and these changes are reversible [16]. Various animal tissues have been used for studies for immunotherapies, transfer of multiple plasmids at the same time, and gene expression's therapeutic level achieved. Electro-transfer is not much efficient with viral vectors but this method has better delivery over chemical and physical techniques [17]. The therapeutic purpose is the main factor for choosing transfection as compared to transduction with viruses. For example, the method of synthetic delivery is better for dose repeating or transient gene expression. For the treatment of missing protein disease, viral vectors are a better choice because they can give gene expression for and longtime stability which then can be integrated with the DNA of the host and start producing protein. Gene expression is more efficient with viral vectors over the synthetic system [18] (**Table 1**).

Commonly in gene therapies abnormal or faulty genes are replaced by the healthy or normal gene. For doing gene therapy, a molecule used to introduce the gene to the patient used to carry DNA known as a vector [17]. Viruses can get into the human body and insert their genome into it, so by taking advantage of their abilities scientist change their genome and insert therapeutic genes. Various viruses are used as vectors for therapy [19].

- **Adenoviruses:** These viruses containing double-stranded DNA and are responsible for multiple human diseases including respiratory, intestine, and infections of the eyes
- **Retroviruses:** Having abilities to synthesize copies of their double-stranded DNA from RNA and can be inserted into the host cell's chromosomes. Retrovirus is also Human Immunodeficiency Virus (HIV)
- **Adeno-associated viruses:** These types of viruses are very small containing DNA of single strands. These viruses can integrate their genome into the chromosome at particular sites
- **Herpes Simplex Viruses (HSV):** These viruses contain DNA of Double strands and having the ability to target neurons cells. Type 1 viruses are also responsible for cold sores because these are human pathogen

Vectors

For inserting desired gene or genetic information to the organism required vehicles known as vectors. There are two types of vectors viral and non-viral. Retroviruses, adenoviruses, and AAV are the most common category of viral vectors that have been used [20]. Various viral vectors such as HCV-1, baculovirus, or vaccinia virus are used less frequently. Non-viral vectors are plasmids, double-stranded and circular, and can replicate in hosts or chemicals. For proper functioning and requirement of the delivery system, these things have to consider such as characteristics of target cells that can achieve by ex vivo, long-lasting expression, and inserted gene size [21] (**Table 2**).

Applications of Gene Therapy

The disease of single gene defect, treated by gene therapy and have a very high rate of success. Gene therapy was approved in late 1993 for curing diseases such as hypercholesterolemia, Gaucher's disease immunodeficiency, and cystic fibrosis [22]. Most studies

Table 1: Protocols for gene therapy.

Countries	Diseases	Target cells	Objective	Release mode
USA	Rheumatoid arthritis	Synovial membrane	For the release of cytokine	Retroviruses
China	Hemophilia B	Skin fibroblasts	Substitution of IX factor	Retroviruses
USA	Gaucher disease	Bone marrow and blood	Substitution of Glucocerebrosidase	Retroviruses
USA	Fanconi anemia	Bone marrow and blood	Release of the complement C gene	Retroviruses
USA	Familial hypercholesterolemia	Liver	Receptors of low-density protein substitution	Retroviruses
England, USA	Cystic fibrosis	Epithelium of respiratory	Substitution of enzymatic	Liposomes and adenoviruses
USA, Sweden, France, Canada	Cancer	Tumor, blood and bone marrow	Stem cell marking	Retroviruses
USA	Cancer	Bone marrow and blood	Chemo-protection	Retroviruses
USA	Cancer	Tumors	Removal of tumors	Cell-mediated transfer, retroviruses and noncomplex DNA
China, France, Italy, Holland, Netherland, USA, Austria, Germany	Cancer	Tumor, blood and bone marrow	Immune function improvement	Cell-mediated transfer, retroviruses, liposome and electroporation
USA	Deficiency of α 1-antitrypsin	Epithelium of respiratory	α 1-antitrypsin substitution	Liposome
USA	AIDS	Blood and Bone marrow	Antigen of HIV inactivation	Retroviruses

Table 2: Characteristics of Viruses used for viral vectors.

Viruses	Physical properties	Viral proteins	Disease in Animals	Genome type and size
Vaccinia virus	Enveloped, 350 by 270 nm rectangle	More than 198 open frame reading virus	Used to treat smallpox	Linear DNA double-stranded 190 kb
Adenovirus	Non enveloped, Diameter about 70-100 nm	More than 20 proteins	Gastroenteritis, cold & conjunctivitis	Linear DNA double-stranded 36 kb
Herpes simplex V1	Diameter about 110 nm	More than 81 proteins	Encephalitis, genital warts & mouth ulcer	Linear DNA double-stranded 152 kb
Baculovirus	Enveloped, 270 by 45 nm	More than 60 proteins	Insect pathogen only	Linear DNA double-stranded 130 kb
Retrovirus	Enveloped and 100 nm	Gag[1], Pol[2], Env[3]	Tumor induction and AIDS	Single-stranded 7-10 kb RNA
Adeno-associated viruses	Non enveloped and 18-26 nm	Rep[4] and Cap[5]	Not known yet	4-7 Linear DNA single-stranded

are looking towards the treatment of fatal diseases such as cancer and AIDS. For Gene therapy treatment various diseases are under consideration such as heart disease, Alzheimer's and Parkinson's disease, and arthritis disease. Under the human genome project, it will be possible to know the location of the entire gene, it helps to find genetic diseases [23]. Criteria of gene therapy treatment selection describe by Eve Nichols:

- The disease is fatal and life-threatening
- Affected cell, tissue, and organ must be identified
- Same, normal functioning genes should be isolated and cloned as the counterpart of a defective gene
- Gene insertion into target cells or tissue like bone marrow will change the process of disease from which tissue was damaged or become faulty
- Gene adequately can be express; it can synthesize new normal proteins [24]

For checking the security of procedures there are various techniques available

Advantages of Gene Therapy

- Gene therapy of Germline is quite successful
- For few genetic diseases, gene therapy is the only way of treatment
- By stopping the insertion of defective genes, risks can be reduced or eliminated for somatic cell therapy for many generations [25]
- For reproductive health needs, medicines should respond to parents in danger of genetic diseases
- Scientific groups should have rights for inquiring and human research freely but within limits

Embryos will be at risk in the technique of germline cell therapy but shortly techniques will be successful and there will be no danger of disposing of the embryo in labs [26].

Conclusion

Gene therapy was used to treat different diseases but initially was not successful. In between the 1970s-1980s two unofficial trials were started and remain unsuccessful, later on in the 1990s officially trials were started but these were also unsuccessful. For the last five years for the treatment of HIV and AIDS therapies successfully have been done. Various drugs have been approved for gene therapy, recombinant DNA is the main technique that has been used. Fatal diseases will be treatable with gene therapy in near future.

References

1 Andrews CD, Huang Y, Ho DD, Liberatore RA (2020) *In vivo* expressed biologics for infectious disease prophylaxis: rapid delivery of DNA-based antiviral antibodies. Emerg Microbes Infect 9:1523-1533.

2 Bae CS, Lee CM, Ahn T (2020) Encapsulation of apoptotic proteins in lipid nanoparticles to induce death of cancer cells. Biotechnol Bioprocess Eng. 25:264-271.

3 Beebe SJ (2020) Pulsed electromagnetic fields for clinical applications. (1st eds.) p:237.

4 Cavazzana MS, Hacein BG, Gross F, Yvon E, Nusbaum P, et al (2010) Gene therapy of human severe combined immunodeficiency (SCID)-X_1 disease. Science. 288 :669-672.

5 Derenne AA, Tassin TH, Nguyen E, Roeck V, Jenart E, et al (2020) Induction of a local muscular dystrophy using electroporation in vivo: an easy tool for screening therapeutics. Sci Rep. 10:1-15.

6 Dermol CJ, Napotnik TB, Rebersek M, Miklavcic D (2019) Short microsecond pulses achieve homogeneous electroporation of elongated biological cells irrespective of their orientation in electric field. Sci Rep. 10:1-17.

7 Ebrahim NV, James AA, Rizvanov, Mukhamedshina Y (2020) Genetic modification of mesenchymal stem cells for neurological disease therapy: what effects does it have on phenotype/cell behavior, determining their effectiveness? Mol Diagn Ther. 24:683-702.

8 Ghosh S, Brown AM, Jenkins C, Campbell K (2020) Viral vector systems for gene therapy: a comprehensive literature review of progress and biosafety challenges. Appl Biosaf. 25:7-18.

9 Jadhav MSD, Rajguru MJR, Momin MHU, Shirsat MK (2020) Gene Therapy-Challenges & Success. Int J Trend Sci Res Dev. 4:870-874.

10 Kaufmann KB, Büning H, Galy A, Schambach A, Grez M (2019) Gene therapy on the move. EMBO mol med. 5:1642-1661.

11 Kumar M, Sharma P, Maheshwari R, Tekade M, Shrivastava SK, et al. (2019) Beyond the blood–brain barrier: facing new challenges and prospects of nanotechnology-mediated targeted delivery to the brain. Nanotechnol.-Based Target Drug Deliv Syst Brain Tumors. 15:397-437.

12 Calle AL, Moreno AD, Bailén GU, Raju J, Antony JS, et al. (2020) Hematopoietic stem cell gene therapy: the optimal use of lentivirus and gene editing approaches. Blood rev. 40:100641.

13 Maged MN, Mohamed M, Lamia HS (2020) Gene therapy for b-thalassemia; new challenges. Int J Sci Healthc Res. 5:158-173.

14 Maihofer JS (2020) rAAV-mediated, hydrogel-bound overexpression of human insulin-like Growth Factor I (hIGF-I) improves the repair of focal cartilage damage and reduces perifocal osteoarthritis-a long-term study in a large animal model of microfracture.

15 Mangion M (2020) Development and characterization of gesifection as a method of nucleic acid delivery in mammalian cells.

16 Misra S (2020) Human gene therapy: a brief overview of the genetic revolution. J Assoc Physicians India. 61:127-133.

17 Muthuraman A, Rishitha N, Mehdi S (2018) Role of nanoparticles in bioimaging, diagnosis and treatment of cancer disorder. Design of nanostructures for theranostics applications. (1st eds.) p:529-562.

18 Patil SR, Zoubi I, Raghuram P, Misra N, Yadav N, et al (2018) Gene therapy: a comprehensive review. Int Med J. 25:361-364.

19 Rodrigues RC, Garcia RL, Baptista PV, Fernandes AR (2020) Gene therapy in cancer treatment: Why Go Nano?. Pharmaceutics 12:233.

20 Rosenberg SA, Aebersold P, Cornetta K, Kasid A, Morgan RA, et al (2010) Gene transfer into humans-immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. N Engl J Med. 323:570-578.

21 Scollay R (2019) Gene therapy: a brief overview of the past, present, and future. Ann N Y Acad Sci. 953:26-30.

22 Shinde MB, Kajale AD, Channawar MA, Gawande (2020) Vector-mediated cancer gene therapy: A review. GSC Biol Pharm Sci. 13:152-165.

23 Wang D, Gao G (2020) State-of-the-art human gene therapy: part II. Gene therapy strategies and applications. Discov med. 18:151-161.

24 Wolff JA, Lederberg J (2019) An early history of gene transfer and therapy. Hum Gene Ther. 5:469-480.

25 Xu J, Wang B, Li S (2021). Gene and cell therapy. Remington. (23rd eds.) p:463-488.

26 Yother J, Mcdaniel LS and Briles DE (2020) Transformation of encapsulated Streptococcus pneumoniae. J of Bacteriol. 16:1463-1465.