

Gene Therapy, Early Promises, Subsequent Problems, and Recent Breakthroughs

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ABSTRACT

Gene therapy is one of the most attractive fields in medicine. The concept of gene delivery to tissues for clinical applications has been discussed around half a century, but scientist's ability to manipulate genetic material via recombinant DNA technology made this purpose to reality. Various approaches, such as viral and non-viral vectors and physical methods, have been developed to make gene delivery safer and more efficient. While gene therapy initially conceived as a way to treat life-threatening disorders (inborn errors, cancers) refractory to conventional treatment, to date gene therapy is considered for many non-life-threatening conditions including those adversely influence on a patient's quality of life. Gene therapy has made significant progress, including tangible success, although much slower than was initially predicted. Although, gene therapies still at a fairly primitive stage, it is firmly science based. There is justifiable hope that with enhanced pathobiological understanding and biotechnological improvements, gene therapy will be a standard part of clinical practice within 20 years.

Introduction

Gene therapy is a form of molecular medicine based on the insertion of a functional gene into cells to correct a cellular dysfunction or to provide a new cellular function.

Gene therapy was originally developed as a strategy to treat inherited monogenic disease such as cystic fibrosis, but now gene therapy is considered for many non-life-threatening conditions including those adversely affecting a quality of life of patient.¹⁻⁵

The idea of gene therapy was introduced by Joshua Lederberg in 1963; but on the whole, research on human genetics did not hasten until the 1980s.⁶⁻⁸

Afterwards, the first FDA approved clinical gene therapy was done by Anderson et al in 1990. In that study, a 4 years old girl with adenosine deaminase (ADA) deficiency was treated by transfecting the ADA gene into her white blood cells, resulting in remarkable improvements in her immune system.^{6,9-11}

In 1990, Rosenberg et al used a retroviral vector to introduce the neomycin resistance marker gene into tumor-infiltrating lymphocytes obtained from 5 patients with metastatic melanoma. Then these lymphocytes were expanded in-vitro and later reinfused into the respective patients.

That was the first practice showed that that retroviral gene delivery was safe and practical, it led to many

other studies.^{6,10,12} Since 1990, more than 1700 clinical trials have been conducted using different techniques for gene therapy.¹³

Development of recombinant DNA technology made gene therapy possible between 1963 and 1990.

In comparison to other traditional medicine, a strong theoretical advantage of gene therapy is the possibility to achieve a long-lasting therapeutic effect in the target tissue by a single administration of the gene without systemic side effects.¹

Gene therapy classification

Gene therapy may be classified into the two types:

Germ line gene therapy

In germ line gene therapy, Germ cells, i.e., sperm or eggs, are modified by the introduction of functional genes, which are integrated into their genomes. This would let the therapy to be heritable and carried on to later generations. If this should, in theory, be highly effective in counteracting genetic disorders and hereditary diseases, many jurisdictions prohibit this for application in human beings, at least for the present, for different technical and ethical reasons.^{14,15}

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Somatic gene therapy

In somatic gene therapy, the therapeutic genes are introduced into the somatic cells, or body, of a patient. Any modifications and effects will be limited to the individual patient only, and will not be inherited by the patient's offspring or future generations. The purpose of somatic gene therapy is to alter the genetic material of living cells in order to achieve therapeutic benefit to the individual.

Somatic gene therapy shows the mainstream line of current basic and clinical research, where the therapeutic DNA transgene (either integrated in the genome or as an external episome or plasmid) is used to treat a disease in an individual.^{14,16,17} Somatic gene therapy is also being evaluated specifically to cure various kinds of cancer. With time, it is optimism that the number of diseases subject to treat by this gene therapy will increase drastically. Numerous trials are being conducted under the supervision of medical experts in the laboratories to make the most of this gene therapy.

Some scientists believe that somatic gene therapy is better than germ line gene therapy. Somatic gene therapy is easier to use in comparison to the germ line gene therapy. Moreover, it does not harm the germ cells and thus the genetic alterations are not passed. It cures the symptoms of the problem and not the root cause.^{18,19}

Strategies in gene therapy

The progression in the field of gene therapy have developed into two different strategies: *ex vivo* and *in vivo* gene therapy.²⁰ *Ex vivo* gene therapy includes the harvesting of cells from a patient followed subsequently by genetically modification *ex vivo* in a laboratory. The genetically modified cells are usually amplified in number, then the transduced cells are returned to the patient.^{12,15,21}

Ex vivo gene therapy is a new therapeutic strategy especially well- suited to targeting a specific organ rather than for treating a whole organism. Therefore the eye and visual pathways make a suitable target for this approach. With blindness still so prevalent worldwide, new strategies to treatment would also be widely applicable and an important advance in improving quality of life. Despite being a relatively new approach, *ex vivo* gene therapy has already gained significant progresses in the treatment of blindness in pre-clinical trials. Particularly, advances are being taken in corneal disease, glaucoma, retinal degeneration, stroke and multiple sclerosis through genetic re-programming of cells to replace degenerate cells and through more refined neuroprotection.²²

But *ex vivo* gene therapy is far less useful when the target organ is internal such as the lung, heart or brain. The lack of immune response is an apparent advantage of *ex-vivo* gene therapy.²³

In vivo gene therapy

Ex vivo gene therapy was not successful to treat internal disorders, because of that the concept of *in vivo* gene

therapy was established. *In vivo* gene therapy is a strategy in which genetic material usually in the form of DNA, is applied to modify the genetic repertoire of target cells for therapeutic goals.

This technology is now being developed in clinical trials as a treatment for hereditary disorders, and is also being considered as a potential treatment of acquired diseases, including atherosclerotic arterial disease, restenosis after vascular interventions, and cardiacallograft rejection.²⁴⁻²⁹

Vectors

There are two general approaches to deliver genes into a cell: viral and non-viral.

Gene delivery mediated by viral vectors is referred to as transduction and gene delivery mediated by non-viral vectors is referred to as transfection.

Viral vectors are highly efficient at introducing genes but can create some safety risks.

Non-viral vectors are remarked to be much safer than viral vectors, but at present, they are somehow inefficient at transferring genes.³⁰⁻³³

Viral systems

Viral vectors, which can deliver genetic materials into host cells, are biological systems derived from naturally developed viruses. Viruses used in gene therapy have been modified to enhance safety, increase specific uptake, and improve efficiency.³⁴⁻³⁶

Viral vectors are derived from viruses with either RNA or DNA genomes and are shown as both integrating and non-integrating vectors.

The former promises of lifelong expression of the deficient gene-product.

Efficient gene transduction can also be attained from vectors that are maintained as episomes, particularly in non-dividing cells.

The viral vectors include RNA virus such as retrovirus and DNA virus such as adenovirus, Herpes simplex virus and Adeno-associated virus (AAV), and poxvirus (vaccine virus).³⁷

The most commonly used DNA viral vectors are based on adenoviruses and AAVs. Though, there are some drawback in the safety and toxicity of these vectors and the size of the transfected genetic material.

Therefore, great warning should be exercised when using viral vectors for the treatment of human diseases, and this topic should be investigated further.^{34,36}

Adenoviral vectors

Adenoviral vectors are belonged to the family of adenoviridae.

Adenoviral vectors are DNA viruses, can be produced in high titers, and efficiently transfer transgene into both dividing and non-dividing cells. Human cells are suitable candidate for adenoviral mediated gene delivery. They remain episomal and direct transient gene expression. Cell division causes fast loss of transgene. The infectivity and expression of adenoviral vector is dependent on the expression of adenovirus

specific receptors on the desirable cells and immune response.

The adenovirus envelope fiber protein is important in first attachment and penton protein is mediated in virus internalization.

The adenoviral vectors are currently used are highly immunogenic, so the short term expression of transgene is observed. In addition to elicit inflammatory and toxic reaction in the host, immune response may decrease gene delivery efficiency by omitting transduced cells and limiting the readministrations by generation of neutralizing antibodies. In some disease such as cancer, immunological reactions may be beneficial for the host.

Adenoviral vectors are employed, where the high level of transgene expression is required, such as pathological condition like cancer and angiogenesis.³⁶⁻⁴¹

Adeno-associated virus

Adeno-associated viruses (AAVs) are human parvoviruses that normally require a helper virus, such as adenovirus, to mediate a productive infection. Most studies to date have focused on AAV-2. There is no known disease associated with AAV infection making it an ideal candidate for gene therapy. AAV vectors have been shown to transduce cells both through both episomal transgene expression and by random chromosomal integration.⁴²⁻⁴⁵

Retroviral and Lentiviral Vector

Retroviral and Lentiviral vectors are RNA-viruses and belong to the family of retroviridae. Retroviral vectors are based on Molomy murine leukemia virus (MoML) and they have been used in many clinical trails for the treatment of cancer, inherited and acquired monogenic disorders, and AIDS.⁴⁶

Lentiviral vectors are based on human immunodeficiency virus-1 (HIV-1), non-human immunodeficiency (SIV), or feline immunodeficiency viruses (FLV). Lentiviral Vectors are efficient and stable gene transfer tool to various cells like stem cells. Minimizing the possibility of recombination among various viral genetic elements has increased the safety of Lentiviral vectors. This has been gained by involving less than %5 of the viral genome into the vector and generating self-inactivating vectors.⁴⁷⁻⁴⁹

Retroviral and Lentiviral vectors could lead to a stable integration of the transfected gene into the host genome and a long lasting expression of the transgene. A restriction of the Retroviral vectors is their relatively low titer, which decrease in vivo gene transfer efficiency. Retroviral vectors need to cross the nuclear membrane for integration and can only infect dividing cells. This is a limitation, so the Retroviral vectors are not suitable for non-dividing cell.¹

Non-viral system

The limitation related to viral vectors have Inspired researchers to emphasize on non-viral systems. Several

methods have been developed for the Non-virus-mediated delivery of genetic materials including non-viral vectors.

Non-viral vectors are safe, can be constructed and modified by simple methods, and show high gene encapsulation ability.

The non-viral vectors consist of naked DNA delivered by injection, liposomes (cationic lipids mixed with nucleic acids), nanoparticles, and other ways.

Though non-viral vectors can be produced in relatively large scale and are likely to present minimum toxic or immunological problems, now they suffer from inefficient gene delivery. Furthermore, expression of the foreign gene tends to be transient, precluding their application to many diseased states in which sustained and high-level expression of the transgene is needed.^{33,36}

It is likely that future gene therapy protocols will use new innovations to improve on the efficiency of non-viral vector systems, often building upon observations from viral vector transduction. Non-viral vectors are remarked to be much safer than viral vectors, but at present, they are somehow inefficient at transferring genes.

Enhancement in efficiency have made naked DNA gene delivery a viable method for gene therapy.^{37,50}

Evolution in non-viral gene delivery include

- Injection of naked DNA in muscle causes in-vivo cell transfection available.
- Electroporation increases uptake of injected plasmid DNA into muscle and skin.
- Intravascular transfer of plasmid DNA results in a very effective gene Delivery to hepatocytes.
- Tail vein pDNA delivery is an easy and effective method to liver cells transfect in mice and rats.¹

Physical methods for gene delivery

Over the last decade, physical methods of plasmid delivery have evaluated the efficiency of non-viral gene delivery, in some cases reaching the efficiencies of viral vectors.⁵⁰

Electroporation and other physical methods

Electroporation (EP) is an efficient and easy method to DNA transfer.

This method is relied on the principle that applying electric pulses across the cell membrane creates a trans membrane potential difference, allowing transient membrane permeation and facilitating the insertion of DNA through the destabilized membrane.

EP is a safe and possible treatment approach and has been used to deliver genes into the cells of skeletal muscles, tumors, brain, liver, skin, and other tissues. Among these experiments, 38% are related to cancer treatment.

In addition, genes related to immune response are mostly used in EP mediated tumor treatment.^{51,52}

Ultrasound

Ultrasound can generally be used to deliver ultrasound energy directly to an object and to enhance the delivery of therapeutic drugs and genes.

Ultrasound can enhance gene delivery by altering vascular permeability in a method called sonoporation. Sonoporation has been applied in many tissues, including tumors, and has been used to deliver oligonucleotides and small interfering RNA (siRNA) to tumors.

In the treatment of prostatic tumors, microbubble and ultrasound have been applied to target siRNA to the androgen receptor.⁵³⁻⁵⁵

Hydrodynamic based method

The hydrodynamic based method brought efficient gene delivery and expression by fast injection of a large volume of DNA solution through the tail vein of an animal. But this technique may be harmful for the experimental animal.⁵⁶

Gene gun

Gene gun immunization through the skin is a trustful and reproducible method of DNA vaccine delivery. This method can induce immune response against both infectious diseases causing agent and cancer in animal models.

DNA delivery using this approach requires 205-250 times less DNA than the standard method of intramuscular delivery.

Furthermore, the gene gun immunization is a highly efficient method of achieving antigen presentation.⁵⁷

Clinical Trials

More than 600 clinical trials using gene therapy have been done or are underway, with the enrollment of thousands of patients in the world wide. A portion of these trials (over 70%) are for cancer and are often carried out using end-stage patients. Lots of the clinical trials are in Phase I or II, with less than 1% in Phase III, and so, now there are no commercially approved gene therapy treatments.

Gene delivery into multi potent hematopoietic stem cells has received much attention because of its relevance for a wide variety of human diseases, ranging from hematological disorders to cancer.⁵⁸

Retroviral vectors based on MLV were the first viral vectors to be administered - in a gene therapy trial and continue to be used more.

The first clinical trial tried to treat SCID patients suffering from adenosine deaminase (ADA) deficiency, using retroviral vectors to transduce T lymphocytes.⁵⁹

A main drawback for the field happened in September 1999, when a broadly publicized death resulting from a gene-therapy trial was reported.⁶⁰

Jesse Gelsinger (an 18-year-old man), died in a clinical trial at the University of Pennsylvania which used a modified Ad5 vector to deliver the gene for ornithine decarboxylase, a deficient hepatic enzyme.

Regarding to an investigation by the university, Gelsinger died from a massive immune response to the Ad5 vector.

Luckily for the gene therapy field, less than 1 year after Gelsinger died, the first report of a remarkable success in gene therapy trial was published. In 2000, Cavazzana-Calvo et al in Paris described results from a study involving two children suffering from a severe combined immune deficiency disorder (SCID-XI), which had restricted them to life in an isolated environment.¹¹ These studies used a MOMLV vector to deliver a curative gene (γ c cytokine receptor subunit) into the patients lymphocytes *ex vivo*, and after proliferation of the cells, returned them to the patients both patients were able to leave the hospital and resume normal lives. After that, several other patients were treated and cured in these studies. However, there was a downside of around 11 early patients treated with the MoMLV vector, 3 developed leukemia directly as a result of the gene-transfer procedure.⁶¹

In another clinical study, patients suffering from hemophilia B, a bleeding disorder caused by a deficiency of coagulation factor IX, were treated with AAV vectors expressing human factor IX.

These patients attended in a Phase I trial and received intramuscular injections of AAV vectors. Though only very low levels of secreted factor IX could be detected in the plasma of one patient, the treated patients represented some clinical benefits and a reduced intake of factor.

One of the earliest hopes of gene therapy approaches was the possibility of using viral vectors to either introduce a lethal gene to cancer cells or to boost the immune system so as to recognize the tumor cell as foreign furthermore to using viruses to transfer.^{62,63}

Tumor suppressors, apoptotic inducers, suicide genes, and cytokines, another interesting approach to cancer gene therapy is to harness the lytic action of replicating viruses for tumor-specific killing.⁶⁴

Immune responses to gene therapy vectors

Circumventing the immune response to the vector is a principle challenge with all vector types likely to induce an immune response, particularly those, like adenovirus and AAV, which express immunogenic epitopes within the organism. The first immune response happening after vector delivery emerges from the innate immune system, consisting in a rapid, inflammatory cytokines and chemokines secretion around the administration site.

This reaction is high with adenoviral vectors and somehow null with AAV.^{65,66} It is noteworthy that plasmid DNA vectors, because of CpG stimulatory islets, also stimulate the innate immunity via the stimulation of TLR receptors on leukocytes.⁶⁷⁻⁶⁹

Acquired immune response leading to antibodies production and T lymphocytes activation also occurs within a few days after vector introduction. Capsid antigens are mostly responsible for specific immunity

toward adenoviruses, and are also involved in the response against AAV.

Even, viral gene-encoded proteins can also be immunogenic.⁷⁰⁻⁷³

The pre-existing humoral immunity coming from early infections with wild-type AAV or Adenovirus could avoid efficient gene delivery with the corresponding vectors.^{73,74}

Some parameters like route of administration, dose, or promoter type have been described as critical factors influencing vector immunity.⁷⁵

Conflict of Interest

The authors report no conflicts of interest.

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