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The Critical Role of Aging on Gene Therapy

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Abstract

One of the main goals of aging research is to improve health during aging. In the case of mice, genetic manipulations that shorten or lengthen telomeres result, respectively, in decreased or increased longevity. Gene therapy has become a significant issue in science-related news. The principal concept of gene therapy is an experimental technique that uses genes to treat or prevent disease. Although gene therapy was originally conceived as a way to treat life-threatening disorders (inborn errors, cancers) refractory to conventional treatment, it is now considered for many non–life-threatening conditions, such as those adversely impacting a patient's quality of life. The gene therapy consisted of treating the animals with a DNA-modified virus, the viral genes having been replaced by those of the telomerase enzyme, with a key role in aging. An extent range of efficacious vectors, delivery techniques, and approaches have evolved in the last decade for developing gene-based interventions for diseases. The lack of suitable treatment has become a rational basis for extending the scope of gene therapy. The aim of the present study is to investigate the general methods by which genes are transferred and to give an overview to clinical applications. Maximizing the potential benefits of gene therapy requires efficient and sustained therapeutic gene expression in target cells, low toxicity, and a high safety profile. Gene therapy has made substantive progress albeit much slower than was initially predicted. This study shows that it is possible to develop a telomerase-based anti-aging gene therapy without increasing the incidence of cancer. Aged organisms accumulate damage in their DNA due to telomere shortening finds that a gene therapy based on telomerase production can repair or delay this kind of damage, they add. The present study also describes the basic science associated with many gene therapy vectors and the present progress of gene therapy carried out for various surface disorders and diseases. The results of the study show that by increased pathobiological understanding and biotechnological improvements, gene therapy will be a standard part of clinical practice. Together, these results constitute a proof-of-principle of a role of TERT in delaying physiological aging and extending longevity in normal mice through a telomerase-based treatment, and demonstrate the feasibility of anti-aging gene therapy.

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Introduction

Studies with genetically modified mice, as well as by means of ectopic treatments, have proved that it is possible to ameliorate various age-related parameters (the so-called 'health-span'), which is often accompanied by an increase of life-span. Recently, a significant delay of aging in adult animals was first achieved by a rapamycin-based pharmacological intervention, an agent that reduces the activity of mTOR kinase.

Gene therapy is a normal, functional copy of a gene into a cell in which that gene is not working. Cells, tissue, or even all individuals (when germ-line cell therapy becomes available) modified by gene therapy are considered to be transgenic or genetically modified. After some time, gene therapy could pick out the correction of genetic weakness, eradicate cancerous cells, stop cardiovascular diseases, clog up neurological disorders, and even remove infectious pathogens completely [1].

However, the significant point is that gene therapy is not a novel idea. According to Joshua Lederberg (1963), "We might anticipate the interchange of chromosomes and segments. The ultimate application of molecular biology would be the direct control of nucleotide sequences in human chromosomes, coupled with recognition, selection and integration of the desired genes. It will only be a matter of time . . . before polynucleotide sequences can be grafted by chemical procedures onto a virus DNA." The first clinical study using gene transfer was reported in 1960 [2].

Gene therapy and the use of genomics are related in some respects but they should be distinguished from each other in discovering new medicines and diagnosis techniques.

By obtaining 5 patients with metastatic melanoma, a retroviral vector to transfer the neomycin resistance marker gene into tumor-infiltrating lymphocytes was used by Rosenberg and his colleagues [2]. These lymphocytes then were expanded in vitro and later reinfused into the respective patients. By showing the first study that retroviral gene transfer was protected from or not exposed to danger or risk; it led to numerous other studies. As a matter of fact, more than 900 clinical trials have been approved worldwide since 1989 [3]. The development of recombinant DNA technology made gene therapy be possible between 1963 and 1990.

There are two main types of gene therapy: 1) somatic cell gene therapy, and 2) reproductive or germ-line gene therapy [4].

Somatic cell gene therapy

The cells which related to reproduction indirectly are called gene therapy of somatic cells. They result in changes that are not transmitted to offspring. The introduction of genes in an organ or tissue to induce the production of an enzyme is an instance of it. In general, this alteration does not impact the individual's genetic makeup and it is not transmitted to its descendants. With somatic cell gene therapy, a disabled organ is better able to function normally. This technology has many applications to human health. One variant of somatic cell gene therapy is DNA vaccines, which allow cells of the immune system to fight certain diseases in a method similar to conventional vaccines [5].

Germ-line gene therapy

By introducing of corrective genes into reproductive cells (sperm and eggs) or zygotes, the germ-line cell therapy is produced. The objective of it is to create a favorable or advantageous genetic change that is transmitted to the offspring. When genes are introduced in a reproductive cell, descendant cells can inherit the genes [4].

Via the use of *pluripotent cells*, or cells that can differentiate into any other cell type,

stem cell therapy is produced. Stem cells exist in developing embryos and in some tissues of adults. The goal of this therapy is to regenerate or repair a damaged organ or tissue. Indeed, it is similar to a conventional transplant. Since it uses the individual's own cells, the procedure has a reduced probability of rejection. For example, stem cells differentiated into nerve cells could be used by patients suffering from paralysis, with the goal of helping them recovering movement; or in cases of heart stroke, muscle cells might be used to rejuvenate the cardiac muscles [8]. Moreover, the future may bring the growth of stem cells from an individual's body to produce certain tissues or organs in vitro. Finally, stem cell research could blend gene therapy with genetic engineering to create healthy stem cells that can be used to generate healthy organs and tissue [4,5].

The fundamental advantages of germ-line cell gene therapy are the following:

1. offering the possibility for a true cure of several diseases,

2. being the only way to treat some genetic diseases,

3. extending the benefits for several generations, because genetic defects are eliminated in the individual's genome and, consequently, the benefits would be passed to his or her offspring.

Some of the arguments presented against germ-line cell gene therapy are the following:

1. involving many steps that are poorly understood, and the long-term results cannot be estimated.

2. opening the door for genetic modifications in human traits with great social and ethical implications.

3. being very expensive and it would not benefit the common citizen.

4. being possible to extend the cure to a person's offspring only if the defective gene was directly modified, but probably not if a new gene was added to another part of the genome [4,5].

Applications

Gene therapy is designed for two main purposes: 1) introducing genetic material into cells to recompense for unusual genes, and 2) for making a favorable or advantageous protein. Gene therapy may be able to introduce a usual copy of the gene to restore the function of the protein, if a mutated gene causes a necessary protein to be faulty or missing [6].

When a gene is inserted directly into a cell, it does not usually function. A carrier called a vector is genetically engineered to deliver the gene instead of a gene. Since certain viruses can deliver the new gene by infecting the cell, they are often used as vectors [6].

The viruses are modified and then they used in people. Therefore, they can't cause disease. Some types of virus integrate their genetic material (including the new gene) into a chromosome in the human cell, i.e. retroviruses. Although other viruses introduce their DNA into the nucleus of the cell i.e. such as adenoviruses, the DNA is not integrated into a chromosome [7].

IV can inject or give intravenously the vector directly into a specific tissue in the body, where individual cells take up it. Alternately, a sample of the patient's cells can be removed and exposed to the vector in a laboratory setting. The cells including the vector are then returned to the patient. The new gene delivered by the vector will make a functioning protein, if the treatment is successful [6,7].

Researchers must overcome a lot of technical challenges before gene therapy will be a practical approach to treating disease. For instance, scientists must discover more efficient solutions to deliver genes and target them to

particular cells. They must also assure that the body controls the exactly new genes.

Approaches to gene therapy

Based on [3], there are broadly two approaches to introduce genes into a cell: 1) viral vectors, and 2) nonviral vectors. *Viral vectors* have been used in ~70% of the clinical trials to date. Although viral vectors are extensively efficient at transferring genes, they can create some safety risks. Gene transfer mediated by viral vectors is referred to as transduction. Even though it is believed that *Nonviral vectors* are less dangerous than viral vectors, they are less efficient at transferring genes now. Gene transfer mediated by nonviral vectors is referred to as transfection.

Viral vectors have the advantage of achieving much efficient gene transfer in vivo. Although replication deficient vectors are used, viral vectors still pose important safety problems. The two most common viral vectors used in clinical trials have been those derived from a serotype 5 adenovirus (Ad5; ~26%) and Moloney murine leukemia virus (MoMLV; ~28%), a retrovirus. MoMLV vectors target dividing cells with a reasonably high degree of efficiency. Importantly, they also lead to stable gene transfer because they integrate randomly into chromosomes of the target cell. A basic disadvantage of MoMLV vectors is the risk of insertional mutagenesis caused by the integration of the retroviral genome into the host genome [10,11]. In addition, since retroviral vectors require dividing cells for successful transduction, they some extent are harmful for targeting gene transfer to well-differentiated, quiescent cell types, such as in epithelial tissues. Ad5 vectors have the ability of transduction of both dividing and nondividing cells and make highly efficient gene transfer easier. The other significant point is that Ad5 vectors only too rarely integrate into a chromosome, that is, they exist in a target cell nucleus in an epichromosomal location. Thus, if the target cell divides, only one daughter cell will receive the transferred gene, and with subsequent cell-division cycles, the gene will be dramatically diluted. The major disadvantage of Ad5 vectors is that they induce a potent host-immune response. It is also important to recognize that different viral vectors will vary in their ability to transduce different cell types. Often, this reflects the presence or absence of cell membrane receptor proteins that mediate viral entry into the target cell [11].

Problems

Substantial attention and worthwhile promise has been given to the gene-therapy since the first clinical gene-therapy trial was conducted [2]. There has been considerable public- and private-sector investment, as well as increasingly higher levels of research activity. A large body of preclinical animal model studies has provided proofs of concept for multiple potential clinical applications. In addition, fundamental developments have been made in understanding vector biology and improving vector design and production.

In spite of what mentioned above, clinical progress has been slow and a main setback for the field occurred in September 1999, when a broadly publicized death resulting from a gene-therapy trial was reported [8]. Jesse Gelsinger, an 18-years 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. Based on a study of the university, Gelsinger died from a huge immune reaction to the Ad5 vector. This broadly publicized case resulted in congressional and Food and Drug Administration (FDA) hearings on the conduct of clinical gene-therapy trials as well as a transient hold, subsequently lifted, on all adenoviral-vector

clinical trials. A study by the FDA discovered massive possible violations in the way that this clinical trial had been performed and monitored. After 5 years of studies, the case was settled in February 2005. Because of the Gelsinger case, gene therapy experienced a great phase of criticism and skepticism. Obviously, some mistakes were made in that certain clinical trial, and as a suitable conclusion, all gene-therapy trials are now subject to much tighter regulation by the National Institutes of Health (NIH) and FDA.

Approximately 1 year after Gelsinger's death, the first report of a considerably desired gene therapy trial was published for the gene therapy field. Cavazzana-Calvo and her colleagues, in 2000, in Paris elaborated results from a study in which two children suffering from a severe combined immunodeficiency disorder (SCID-XI), which had confined them to life in an isolated environment [9].These researchers used a MoMLV vector to transfer a curative gene (γc cytokine receptor subunit) into the patients' lymphocytes ex vivo, and after amplification of the cells, returned them to the patients. Both patients had the ability to leave the hospital and continue usual lives. Afterwards, some other patients were treated and cured in these studies evidently. However, there was a drawback. 3 out of 11 early patients cured via the MoMLV vector were developed leukemia directly as a result of the gene-transfer process [10]. The MoMLV vector had integrated apparently in a nonrandom manner near the LM02 (LIM domain only 2) gene in all 11 patients. This integration activated the LM02 gene. Leukemia was the result of it. The patients were cured for the leukemia, and a great, collaborative scientific attempt began to understand what mechanisms impact on MoMLV integration.

Conclusions

The idea of gene therapy has been around for 20 years; however, the procedure has been drawing a great deal of interest and curiosity through the world. The first trials generated great expectations within the scientific community. Although there have been several disappointments, many believe that it is just a matter of time before the technical and scientific details are mastered and the procedures become routine. This study is being advanced worldwide.

Another promising result from gene therapy of stem cell research has been reported in type-B hemophilia patients at the Children's Hospital in Philadelphia and at Stanford University, where patients treated with gene therapy presented a reduction in the period for blood coagulation. ADA deficiency, a disease caused by a defective gene for the ADA enzyme present on human chromosome 20 has been a focus for gene therapy in many institutions. In one of the cases, several patients treated with the corrective gene were able to reconstitute their immune systems and are living normal lives out of the isolated bubbles that are needed to maintain an environment free from microbes. The patients started to produce a correct ADA enzyme after receiving the gene therapy.

The SCID-XI trial likely reflects the path that gene therapy will follow during the next 1 to 2 decades: success, but with some complications. The potential use of this therapy to cure other more complicated diseases, such as cancer and coronary diseases, also seems promising. Gene therapy is still in its infancy, but it is believed that as it matures, it will become an effective treatment for the myriad of genetic diseases that affect humanity.

These experiences add further credence to the general viewpoint offered by Leiden in a 1995 editorial that gene therapy is a field in its infancy, and despite some pitfalls, it is well

grounded in fundamental scientific principles with real clinical promise for the future [11].

References

1. Lister Hill National Center for Biomedical Communications, 2014. U.S. National Library of Medicine. National Institutes of Health. Department of Health & Human Services.
2. Rosenberg, S. A., Aebersold, P., Cornetta, K., Kasid, A., Morgan, R. A., Moen R., Karson, E. M., Lotze, M. T., Yang, J. C., and Topalian, S. L. (1990). Gene transfer into humans—immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. *N Engl J Med* **323**, 570-78.
3. Edelstein, M. L., Abedi, M. R., Wixon, J., and Edelstein, R. M. (2004). Gene therapy clinical trials worldwide 1989-2004—an overview. *J Gene Med* **6**, 597-602.
4. Pearson Education, 2003. This is a sample chapter of Understanding Biotechnology. ISBN: 0-13-101011-5. For the full text, visit http://www.phptr.com. All Rights Reserved.
5. Genetics Home Reference - http://ghr.nlm.nih.gov/. Handbook Gene Therapy. Published September 29, 2014.
6. Braddon, V. R., Chiorini, J. A., Wang, S., Kotin, R. M., and Baum, B. J. (1998). Adenoassociated virus-mediated transfer of a functional water channel into salivary epithelial cells in vitro and in vivo. *Hum Gene Ther* **9**, 2777-85.
7. Cavazzana-Calvo, M., and Fischer, A. (2007). Gene therapy for severe combined immunodeficiency: are we there yet? *J Clin Invest* **117**, 1456-65.
8. Raper, S. E., Chirmule, N., Lee, F. S., Wivel, N. A., Bagg, A., Gao, G. P., Wilson, J. M., and Batshaw, M. L. (2003). Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer. *Mol Genet Metab* **80**, 148-58.
9. Cavazzana-Calvo, M., Hacein-Bey, S., de Saint Basile, G., Gross, F., Yvon, E., Nusbaum, P., Selz, F., Hue, C., Certain, S., Casanova, J. L., Bousso, P., Deist, F. L., and Fischer, A. (2000). Gene therapy of huma severe combined immunodeficiency (SCID)-X1 disease. *Science* **288**, 669-72.
10. Fischer, A., Abina, S. H., Thrasher, A., von Kalle, C., and Cavazzana Calvo, M. (2004). LM02 and gene therapy for severe combined immunodeficiency. *N Engl J Med* **350**, 2526-27.
11. Leiden, J. M. (1995). Gene therapy—promise, pitfalls, and prognosis. N Engl J Med 28, 333, 871-73.