

Updates on Current Advances in Gene Therapy

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ABSTRACT

Gene therapy is the attempt to treat diseases by means of genetic manipulation. Numerous challenges remain to be overcome before it becomes available as a safe and effective treatment option. Retroviruses and adenoviruses are among the most commonly used viral vectors in trials. The retrovirus introduces the gene it carries into the target cell genome while the adenovirus introduces the gene into the target cell nucleus without incorporating it into the target cell genome. Other viral vectors such as adeno-associated viruses, pseudotyped viruses and herpes simplex viruses, are also gaining popularity. Proposed non-viral methods for gene transfer include physical methods and the employment of chemical vectors (lipoplexes, polyplexes and inorganic nanoparticles). Recent studies have investigated potential applications of gene therapy in correcting genetic diseases, treating malignant disorders and for treatment of other diseases. Trials on gene therapy for SCID and Leber's congenital amaurosis have achieved considerable success, but the widely publicized adverse reaction in X-linked SCID patient receiving gene therapy raised concerns for safety profile of gene therapy.

For that, several methods of improving safety and efficacy of gene therapy have been proposed. At present, the three main gene therapy strategies for treatment of cancer are application to oncolytic viruses, suicide-gene therapy and gene-based immunotherapy. Gendicine, the first approved anticancer drugs based on the use of gene therapy principle, is based on the use of oncolytic viruses. More evidence for wider clinical applications of gene therapy are expected as more gene therapy studies progress from the preclinical phase to clinical trial.

Keywords: Adenovirus, gene therapy, nonviral vectors, oncolytic viruses, retrovirus, suicide gene therapy.

Actualizaciones Acerca de los Adelantos Presentes en Terapia Genética

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RESUMEN

La terapia genética es el intento de tratar enfermedades por medio de la manipulación genética. Quedan aún numerosos retos que superar antes de que esté tipo de tratamiento se encuentre disponible como una opción segura y eficaz. Los retrovirus y los adenovirus se hallan entre los vectores virales más comúnmente utilizados en ensayos: el retrovirus introduce el gen – del cual es portador – en el genoma de la célula de destino, mientras el adenovirus introduce el gen en el núcleo de la célula de destino sin incorporarlo al genoma de la célula de destino. Otros vectores virales tales como los virus adenoasociados, los virus pseudotipados, y los virus del herpe simple, también están ganando popularidad. Los métodos no virales propuestos para la transferencia de genes incluyen tanto métodos físicos como el empleo de vectores químicos (lipoplexes, polisomas y nanopartículas inorgánicas). Estudios recientes han investigado las aplicaciones potenciales de la terapia genética en la corrección de las enfermedades genéticas, el tratamiento de los trastornos malignos y para el tratamiento de otras enfermedades. Los ensayos de terapia genética para SCID y la amaurosis congénita de Leber han logrado un éxito considerable, pero la reacción adversa ampliamente divulgada en el caso de los pacientes con SCID ligada al cromosoma, que recibían terapia génica, causó preocupación en cuanto

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al perfil de seguridad de la terapia génica. Por esa razón, se han propuesto varios métodos para mejorar la seguridad y la eficacia de la terapia génica.

En la actualidad, las tres estrategias principales de terapia de genes para el tratamiento del cáncer son la aplicación de virus oncolíticos, la terapia con gen suicida, y la inmunoterapia genética. La gendicina, el primer medicamento anticancerígeno aprobado, basado en el uso del principio de la terapia génica, se basa en el uso de virus oncolíticos. Se esperan más evidencias a favor de aplicaciones clínicas más amplias de la terapia génica, a medida que más estudios de terapia génica progresan de la fase preclínica a la fase de ensayo clínico.

Palabras claves: Adenovirus, terapia génica, vectores no virales, virus oncolíticos, retrovirus, terapia con gen suicida

West Indian Med J 2011; 60 (2): 189

INTRODUCTION

Gene therapy is the attempt to treat diseases by means of genetic manipulation. More than two decades have elapsed since Blaese *et al* carried out the first human gene therapy clinical trial (1). Recently, gene therapy researchers successfully demonstrated clinical applicability of gene therapy in several diseases, including the treatment of Leber's congenital amaurosis (2) and Severe Combined Immunodeficiency [SCID] (3). However, despite these encouraging trial results, a number of challenges remain to be overcome before gene therapy become available as a safe and effective treatment option. Currently, nearly all gene therapy applications in human are experimental and the United States Food and Drug Administration (US FDA) has yet to approve any gene therapy product for sale (4). This article summarizes the concepts behind gene therapy, its limitation and recent developments of gene therapy research.

Basic Principles of Gene Therapy

In the majority of clinical trials, gene therapy involves inserting the "functional" genes into the patient's cells to replace the "defective" genes. Other genetic manipulation approaches include repairing the defective genes, turning off the defective genes or simply introducing the normal genes into the target cells.

In order to deliver the intended gene into the target cells, a vehicle for carrying genes, known as a vector, is required. Two classes of vectors are commonly used for gene transfer: viral vectors and non-viral.

Viral vectors

Table 1 compares the main properties of more commonly used viral vectors in gene therapy trials. At the present, retroviruses and adenoviruses are among the most commonly used viral vectors in gene therapy trials (5).

A retrovirus is a RNA virus that is well known for its ability to integrate itself into its host's genome during its course of infection. After the virus gains entry into the host cell, the reverse transcriptase enzyme it carries, will produce a DNA copy of viral positive mRNA, and then another viral enzyme, integrase, will integrate the DNA copy into the host genome.

To introduce specific gene material into the target cell using a retrovirus as a vector, one inserts the intended gene into the retroviral gene sequence, and then infects the target cell with the modified retrovirus. The retrovirus would then introduce the gene it carries into the target cell genome. Once the intended gene is incorporated into the host cell's genome, all offspring cells from the host cell's replication are expected to contain the inserted gene. This property of retroviruses made it a preferred vector for gene therapy treatments that require long-term, sustained gene expression. However, as retroviruses can insert the gene in any position in the host's genome, it could disrupt a gene if inserted in the middle of a certain gene. Retroviral insertion in proximity to certain cellular pro-oncogenes could also induce uncontrolled cellular proliferation leading to cancer (6).

While retrovirus infection would result in the integration of the gene it carries into the host genome, an

Table 1: Comparison of commonly used viral vectors in gene therapy

Virus	Gene material	Packaging capacity	Chromosome Integration	Key properties
Retrovirus	RNA	8 kb	Yes	Infects only dividing cells, persistent gene expression
Adenovirus	dsDNA	30 kb	No	Efficient short term gene expression
Adeno-associated virus	ssDNA	5 kb	No	Carry small amount of gene material
Lentivirus	RNA	8 kb	Yes	Infects both dividing and quiescent cells, persistent gene expression
Herpes simplex virus-1	dsDNA	40 kb	No	Strong tropism for neurons

adenovirus would introduce the gene it carries into the host cell's nucleus without incorporating the gene into the host genome. As a result, the introduced gene would be transcribed and expressed on the host cell for a certain period of time, but will not be present in the genome of the host cell's offspring after the host cell undergoes replication. This property of adenovirus made it useful in certain applications of gene therapy (7).

An adeno-associated virus is a recombinant virus which is currently under investigation as a possible substitute for adenovirus. Like an adenovirus, it does not integrate into the host genome. It has the advantages of being less antigenic, able to deliver genes into quiescent cells (including neurons, hepatocytes and myocytes) and capable of inducing long term gene expression by inserting at a specific site at chromosome 19. Its major drawback is that it can only carry a limited amount of DNA (8).

Pseudotyped viruses and herpes simplex virus are also commonly used viral vectors in recent clinical trials. Pseudotyped viruses are viruses coated with specific enveloped proteins. Such coating provided the virus with the ability to attach and gain entry to cells previously not susceptible to infection. The most popular pseudotyped virus for use in trials is the vesicular stomatitis virus (VSV) G-pseudotyped lentivirus. Lentivirus itself is a good transfer agent which could insert large genes, and the VSV-G coating of lentivirus render it possible to infect nearly all types of cells. Trials had also been carried out using specific coatings, which enable the coated virus to infect only specific cell population (9). Meanwhile, Herpes simplex virus is of particular interest for nervous system gene therapy investigators, as its ability to infect neurons made it useful for gene transfer into the nervous system (10).

Non-Viral vectors

Over the past decade, numerous non-viral methods for gene transfer had been proposed, including physical methods and the employment of chemical vectors. In physical methods, researchers attempt to enhance gene delivery by exerting physical forces. Methods such as needle and jet injection, hydrodynamic gene transfer, gene gun delivery, electroporation and sonoporation had been described. Meanwhile, chemical vectors currently in use include cationic lipids (forming lipoplexes upon mixing with DNA), cationic polymers (forming polyplexes upon mixing with DNA) and inorganic nanoparticles. These non-viral vectors offer several advantages over viral vectors: ease of large scale production, low immunogenicity, low toxicity and potential for more tissue specificity. However, despite recent technological advances, the transfection efficiency of non-viral vectors is still low when compared to viral vectors. Nonetheless, recent studies had shown that non-viral vectors indeed hold great promise for their future development (11).

Different vectors, with different properties, are preferentially employed in different scenarios. However, re-

gardless of vectors used, there are two general approaches for gene delivery by vectors in gene therapy. In the *ex vivo* approach, the intended gene is delivered into target cells (previously obtained from the recipient) *in vitro*, and then the modified target cells are transplanted back to the recipient. While for the *in vivo* approach, the intended gene is delivered directly into the target cells in the recipient. Recent trials revealed that prior host conditioning, such as myeloablative conditioning that deplete host marrow cells before T-cell transplantation, could particularly increase the success rate of the *in vivo* approach. Both *in vivo* and *ex vivo* approaches had been utilized in both preclinical and clinical trials, with variable degree of success for treating different diseases.

Recent Application of Gene Therapy For Genetic Diseases

Recent studies had investigated many of the potential applications for gene therapy. Many of these studies explored the potential role of gene therapy in correcting genetic diseases or treating malignant disorders, but there are also other studies that researched the role of gene therapy in treating other diseases such as cardiovascular diseases or HIV/AIDS. Gene therapy for correcting genetic disorders had seen both successes and setbacks over the last few years. Studies for SCID and Leber's congenital amaurosis are among the most widely publicized clinical trials, but progress was also made in treatment of other diseases as well.

In order to be clinically practical, an ideal gene therapy technique for correcting genetic diseases should have an efficient and specific gene delivery, capable of long-term gene correction, and possessing relative high safety profile (has minimal adverse effects and antigenicity).

Capability of long-term gene correction is a key feature of gene therapy as a treatment result in only limited or transient correct gene expression would unlikely bring significant long term improvement for genetic diseases such as SCID. The inability to sustain correct gene expression had once plagued earlier *in vitro* and animal studies, (12) but later studies managed to overcome some of the limitations and had considerable success in achieving sustained gene expression (13–16).

Recently, the widely publicized adverse reaction in patients receiving X-linked SCID raised concerns for safety profile of gene therapy. Despite succeeding in maintaining long-term gene correction and immune restoration in the majority of SCID patients in the clinical trials, Hacein-Bey-Abina *et al* reported that a T-cell lymphoproliferative syndrome developed within 2 to 5 years after the procedure in 5 children (17, 18). Investigations have implicated insertional mutagenesis through upregulation of cellular proto-oncogenes as induction of the syndrome (19).

Following the events, several methods of improving safety and efficacy of gene therapy had been proposed, including safer vectors, safer protocols, and use of direct *in situ* gene repair to eliminate the possibility of random gene insertion. Safer vectors had been studied and includes self-

inactivating retroviral vectors that do not interact with neighbouring cellular genes (20) and vectors that have better integration site selection [such as lentivirus] (21). Busulfan was once a frequently used agent for facilitating gene integration by cyto-reduction of marrow stem cells before gene therapy (16) but less toxic alternatives such as monoclonal antibodies had been studied (22). Physiological promoters (23) and chromatin insulators (24) have also been shown to decrease the risk of oncogenesis.

SCID

Gene therapy for SCID has seen major progression since the first clinical trial in 1990. Technological advances that enabled sustained gene expression of gene inserted by vector had greatly enhanced the therapeutic potential of gene therapy for SCID. Clinical trials had demonstrated clear therapeutic benefits of gene therapy in treatment of both X-linked SCID and SCID caused by ADA deficiency (3,13–16). Even though some of the X-linked SCID patients receiving gene therapy suffered from a T-cell leukaemia like syndrome, all but one of the patients responded well to conventional anti-leukaemia treatment. The benefits of gene therapy for X-linked SCID still appeared to outweigh its potential risk (17–19).

Eye diseases

The successes of several clinical trials to restore visual function in patients suffering from Leber's congenital amaurosis, a genetic disease that leads to blindness by adulthood, had been one of the most encouraging news for gene therapy (25). A recent follow-up at one-year of therapy shows marked vision improvement in some patients (2). It was also indicated that the treatment has a relative good safety profile (26).

Patients with certain other eye diseases might also benefit from gene therapy. A phase I study has demonstrated some therapeutic effects of anti-angiogenic cytokine pigment epithelium-derived factor (PEDF) in treating age-related macular degeneration (27). Recently, Mancuso *et al* reported the success of the team in producing trichromatic colour vision in adult red-green colour blind monkeys by subretinal injection of adeno-associated virus containing a L-opsin gene. The study implicates the future potential of gene therapy in the treatment of adult colour blindness or even other adult vision disorders (28).

Other genetic diseases

There are many other genetic diseases proposed for treatment using gene therapy, including certain muscular dystrophies (29), cystic fibrosis (30), alpha-1-antitrypsin deficiencies (31), Huntington's disease (32), lysosomal storage diseases (33), chronic granulomatous disease (34), ornithine transcarbamylase deficiency (35), junctional epidermolysis

bullosa (36) and haemophilia (37). Gene therapy has demonstrated potential therapeutic effects for many of these diseases in preclinical studies, but more human trials are needed to provide evidence for treatment of these diseases by gene therapy.

Recent Application of Gene Therapy For Cancer

Gene therapy for treatment of cancer is a rapidly growing field. By December 2009, it was estimated that 64.5 % of all gene therapy clinical trials worldwide were dedicated to the treatment of cancer (38). At present, there are three main gene therapy strategies proposed for treatment of malignant disorders.

Oncolytic viruses are employed in the first strategy. In this strategy, researchers attempt to induce the death of malignant cells by introducing specific genes into the malignant cells by oncolytic viruses. Studies had demonstrated oncolytic abilities on both natural occurring (39) and recombinant viruses (40). Gene replacement strategy, which attempts to induce oncolysis by delivery of p53 gene (a tumour suppressor gene) into tumour cells by a recombinant viral vector, is of particular interest to cancer gene therapy researchers. In October 2003, Chinese State Food and Drugs Administration (SFDA) approved Gendicine, a recombinant human 5-adenovirus carrying a human wild-type p53 expression cassette, as the first approved anticancer drug based on gene therapy principle (41, 42).

Suicide-gene therapy, or prodrug activation therapy, is another strategy to treat cancer by means of gene therapy. It attempts to treat tumour by delivery of gene coding for enzymes that metabolize prodrugs into locally active chemotherapeutic agents. Recent human clinical trials focussed on delivery of herpes simplex thymidine kinase (Hstk) gene to activate prodrug acyclovir, ganciclovir or valacyclovir (43). The role of the bacterial cytosine deaminase (cd) gene, which increases susceptibility to 5-fluorocytosine and mammalian cytochrome P450 2B (CYP2B) gene, which increases susceptibility to cyclophosphamide and ifosfamide, are also actively experimented (44).

The third strategy is known as the gene-based immunotherapy. As its name suggested, the therapy seeks to enhance host immunity response against tumour cells. One method to enhance such immune response is by tumour lysate therapy, in which researchers feed tumour antigen to dendritic cells, thereby increasing the immune response of dendritic cells against tumour. Currently, a "dendritic cell vaccine" for recurrent prostate cancer has been recommended for approval by US FDA (45). Some other researchers attempt to increase dendritic cells immune response by fusion of dendritic cells with tumour cells (46). Immunotherapy that enhances T-cell functionality against tumour cells are also investigated in several trials (47).

Role of Gene Therapy in Treatment of Other Diseases

Coronary and peripheral artery diseases

The most recent gene therapy study for treatment of coronary artery disease and peripheral artery disease are aimed at patients who were unable to receive conventional therapies. In most of the studies, stimulation of angiogenesis was attempted by injection of angiogenic stimulating gene such as vascular endothelial growth factor (VEGF) gene or fibroblast growth factor (FGF). However, currently, none of the major gene therapy clinical trials for coronary artery disease or peripheral vascular diseases had demonstrated significant therapeutic benefit (48–51).

Parkinson's disease and Alzheimer's disease

Gene therapy for Parkinson's disease is among the most successful gene therapy studies for neurologic diseases. Currently, three approaches for treating Parkinson's diseases by gene therapy have been studied in clinical trials. The first approach attempts to ameliorate Parkinson's disease by transmitting the gene for glutamic acid decarboxylase into the subthalamic nucleus (52). The second approach seeks to prevent degeneration of nigral neurons by delivery of the gene for neurturin putamenal cell bodies (53). So far the preliminary human trials show beneficial results for both approaches. In the third approach, researchers transmitted the gene for aromatic L-amino acid decarboxylase into the striatum to promote conversion of L-dopa into active dopamine. Investigators had successfully demonstrated clinical improvement in MPTP-lesioned primate treated by this approach (54).

Gene therapy for Alzheimer's disease is attempted by delivery of the Nerve Growth Factor (NGF) gene into human central nervous system. Nerve Growth Factor itself is a nervous system growth factor thought to be able to stimulate the function of cholinergic neurons. While previous human *ex vivo* trials for this approach had shown some trophic effects and potential slowing of cognitive decline after treatment, (55) recent *in vivo* trials utilizing AAV and vector (which are simpler and potentially more effective) are still in progress (56). Possibilities of treating Alzheimer's disease using other factors or other vectors, such as lentiviral vectors, are also under investigation (57).

Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS)

In gene therapy for HIV/AIDS, certain transgenes are transferred into haematopoietic stem cells (58) or into T-cells, (59) in order to confer specific protection against HIV infection to these cells. The transgene could function by inactivating HIV-1 protein, or simply creating an environment unsuitable for HIV-1 replication. Both clinical T cell and haematopoietic stem cell gene transfer trials have demonstrated promising results.

CONCLUSION

Clinical trials have demonstrated beneficial effects of gene therapy in the treatment of certain genetic diseases, and it also holds great promise in treatment of cancer. New protocols had also made the therapy safer than before. More evidence for wider clinical applications of gene therapy are expected as more gene therapy studies progress from the preclinical phase to clinical trial.

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