



Gene Therapy in Paediatrics - A Review

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ABSTRACT

Genes are the basic physical and functional units of hereditary and are the specific sequences of bases that are carried on chromosomes which encode instruction, ensure the formation of proteins. Deficiency or disorders of genes in cells leads to the genetic disorders resolved with the respected treatment of gene therapies. The different types of viruses used as gene therapy are retroviruses, adenoviruses, adeno-associated viruses, herpes simplex viruses. Gene therapy is a type delivery where therapy carries the promise of cures for many diseases and for types of medical treatment most of us would not have thought possible. With gene therapy, the treatment or elimination of inherited diseases or physical conditions due to these mutations could become a reality. Gene therapy methods have continued to develop rapidly, and many initial limitations that hampered clinical application have been overcome. Thus serious consideration of clinical application of gene therapy is warranted for selected disorders in which the pathogenesis is well defined. Gene therapy is used to paediatrics for treating leukaemia, diabetics, arthritis, cystic fibrosis etc.

Keywords: Inherited diseases, Leukaemia, Mutations, Viruses.

INTRODUCTION

Gene is composed of strands of a molecule called DNA and is located in single file within the chromosomes. The genetic message is encoded by the building blocks of the DNA, which are called nucleotides. Approximately 3 billion pairs of nucleotides are in the chromosomes of a human cell, and each person's genetic makeup has a unique sequence of nucleotides. This is mainly what makes us different from one another. From this we can get our parents, they go far in determining our physical traits — like the colour of our eyes and the colour and texture of our hair. They also determine things like whether babies will be male or female, the amount of oxygen blood can carry, and IQ. In each cell there are about 25,000 genes. Mutation or change, in any one of these genes can result in a disease, physical disability, or shortened life span. Mutations can be passed from one generation to another, inherited just like a mother's red hair or a father's brown eyes. Mutations also can occur spontaneously in some cases, without having been passed on by a parent. Gene therapy carries the promise of cures for many diseases and for types of medical treatment most of us would not have thought possible. With gene therapy, the treatment or elimination of inherited diseases or physical conditions due to these mutations could become a reality.

There are mainly two types of gene therapy¹

Somatic gene therapy

Involves introducing a "good" gene into targeted cells to treat the patient — but not the patient's future children because these genes do not get passed along to offspring. In other words, even though some of the patient's genes may be altered to treat a disease, it won't change the chance that the disease will be passed on to the patient's

children. This is the more common form of gene therapy being done.

Germ line gene therapy

Involves modifying the genes in egg or sperm cells, which will then pass any genetic changes to future generations. In experimenting with this type of therapy, scientists injected fragments of DNA into fertilized mouse eggs. The mice grew into adults and their offspring had the new gene. Scientists found that certain growth and fertility problems could be corrected with this therapy, which led them to think that the same could be true for humans. Although it has potential for preventing inherited disease, this type of therapy is controversial and very little research is being done in this area, both for technical and ethical reasons.

Effects of Gene therapy²

Gene therapy is done only through clinical trials, which often take years to complete. After new drugs or procedures are tested in laboratories, clinical trials are conducted with human patients under strictly controlled circumstances. These trials usually last 2 to 4 years and go through several phases of research. In the United States, the U.S. Food and Drug Administration (FDA) must then approve the new therapy for the market place.

The most active research being done in gene therapy for kids has been for genetic disorders such as cystic fibrosis. Other gene therapy trials involve children with severe immunodeficiency such as adenosine deaminase (ADA) deficiency (a rare genetic disease that makes kids prone to serious infection), and those with familial hypercholesterolemia (extremely high levels of serum cholesterol).



Gene therapy in pediatrics mainly in:-

Osteogenesis imperfect

Bone marrow mesenchymal cells can differentiate to a variety of tissues including bone, cartilage, muscle, and fat.¹⁻⁷ Thus, in principle, bone marrow transplantation (BMT) could provide effective therapy for disorders that involve cells derived from mesenchymal precursors. One attractive candidate is osteogenesis imperfecta (OI) or "brittle bone disease," a genetic disorder caused by defects in type I collagen. Briefly, patient length was determined by direct measurement, in triplicate, from crown to heel by the same observer (P.L.G.). Growth velocity is defined as the difference between the first and last measurement of each interval, reported as a percentage of the median growth velocity for age- and sex-matched healthy children. Total body bone mineral content was determined by dual energy x-ray absorptiometry performed on a whole-body scanner

Cancer

The majority of research into applications for regulatable gene expression vectors is in models of cancer. This is due, in part, to inadequacies of current therapies, including radiotherapy and chemotherapy. One strategy commonly employed is to regulate expression of a cytotoxic gene with a promoter that can be activated by ionizing radiation. The first example of successfully utilizing radiation to induce gene expression in a tumour model was described more than a decade ago. It was found that TNF- expression, under the control of the Egr-1 promoter, could be increased in response to ionizing X-ray radiation and this was associated with an improved control of tumour growth in comparison with X-ray radiation alone. Since then, other radiation-sensitive promoters, including VEGF, Rec-A, and WAF-1 promoters, have been investigated in preclinical tumour models. Induction of gene expression by ionizing radiation offers a number of advantages over other inducible systems for treating cancer. These include the ability to control temporal and spatial gene expression within the ionizing radiation field, reducing damage to adjacent, healthy tissue. In addition, certain genes, including TNF- and inducible nitric oxide synthase, have synergistic cytotoxic functions when utilized in combination with ionizing radiation. The anatomy of growing tumours has been exploited by another commonly employed inducible gene therapy strategy. As a consequence of relatively poor vascularization, the centre of most tumours is usually a nutrient-starved and hypoxic environment. Promoters activated by hypoxia or nutrient deficiency have been utilized to drive expression of tumouricidal genes. The glucose-regulated protein 78 promoter was used to express HSV-1 thymidine kinase (TK) and successfully eliminate murine fibrosarcomas. HSV-1-TK was also used successfully to treat hepatocellular carcinoma when expressed by a hypoxic-responsive element/ -fetoprotein promoter. Inducible promoters such as radiation-sensitive or hypoxia-sensitive promoters are highly specific, and

ease of use makes them valuable tools for managing certain diseases. Unfortunately, the application of these promoters is limited and they are not suitable for treating the majority of diseases.

Ischemia

Hypoxia-driven promoters are also of great benefit against other diseases, most notably ischemia. Myocardial ischemia can be a chronic illness with repeated bouts of severe hypoxia and can eventually result in myocardial fibrosis and death. By placing hypoxia-responsive elements within the promoter MLC2v, researchers have developed a tissue-specific hypoxia-responsive promoter expressed predominantly in heart tissue that can induce reporter gene expression by up to 400-fold when expressed by adeno-associated viral vectors. Similar vectors expressing VEGF have been demonstrated to increase angiogenesis, improve cardiac function, and reduce myocardial infarcts in mouse model of ischemia

Diabetes

Diabetes is currently treated by daily injections of recombinant insulin to compensate for a deficiency in either production or function of insulin. Although successful for treating the disease, it can be expensive and can also result in a reduction of quality of life due to daily injections and imposed dietary restrictions. Inducible insulin expression by gene therapeutic vectors would circumnavigate many of the disadvantages of conventional insulin therapy. Researchers have investigated the ability to engineer hepatocytes to express and secrete a functional, modified insulin protein under the control of a rapamycin-responsive promoter. Using adenoviral-associated vectors to infect hepatocytes, gene expression was found to be negligible in the absence of rapamycin, but was potently and reversibly expressed in a mouse model of the disease when treated with rapamycin. In a related study, glucose-responsive elements were used to drive insulin expression in hepatocytes *in vitro* and *in vivo*. The authors found that fasting blood glucose levels were returned to normal in a rat model of diabetes

Arthritis

Arthritis affects 1% of the population and is primarily a disease of the elderly. It is characterized by chronic and painful inflammation at the joints of individuals. This chronic inflammatory response has been used by researchers attempting to treat symptoms of arthritis with inducible gene therapeutic vectors. Although cytokine-responsive promoters were successfully used to drive the expression of reporter genes in a model of arthritis, most current research is now focused on the robust Tet-ON regulatory system for treating this disease

Delivery systems available for gene therapy³

The aim of gene therapy is to introduce therapeutic genes into target cells, leading to efficient and stable expression



of the therapeutic molecules and minimizing any putative adverse inflammatory or cytotoxic side effects. This can be achieved using viral and nonviral vectors.

Important parameters to be considered when choosing a gene therapy vector include⁴:

- (1) Size limitations for insertion of transgenes
- (2) Purity and titre of the vector
- (3) Transduction efficiency
- (4) Ability to infect dividing and/or quiescent cells
- (5) Long-term expression of transgenes
- (6) Integration into the host genome
- (7) The need for cell-type specificity or targeted delivery
- (8) vector-associated toxicity and immunogenicity

Viral vectors

Viruses can easily enter cells and deliver their genetic material into the nucleus of target cells; therefore, they are in most cases more efficient than non-viral delivery systems. Most vectors used for gene delivery are derived from human viral pathogens that have been made non-pathogenic by deleting essential viral genes. They usually have a broad tropism; therefore they can infect and deliver their encoded transgenes to a wide spectrum of cells and/or tissues. The most commonly used viral vectors for gene therapy are adenovirus (Ad), adeno-associated virus (AAV), herpes simplex virus type 1-derived vectors (HSV-1), and retrovirus/lenti virus. The main groups of viral vectors currently being developed for gene therapy applications. These vectors have also been used to encode regulatable gene expression systems. HSV-1, herpes simplex type

Adenoviral vectors⁵

Adenoviruses are a family of DNA viruses characterized by a capsid containing a linear double-stranded DNA genome of 36 kb. Adenovirus infection is initiated when the fiber protein binds to the coxsackie virus and adenovirus receptor on the cell surface. The penton then binds to integrins v3 and v5 on the cell surface, which facilitates viral internalization by endocytosis. Once inside the cell, the Ad virion disassembles, during which the adenovirus hexon capsid protein remains at the nuclear membrane while the viral DNA is released into the nucleus and remains as an episome.

First-generation adenoviral vectors (RAd) have been developed for gene therapy based on human adenovirus type 2 and human adenovirus type 5 and were made replication defective through deletions in the E1 and E3 regions. The transcriptional cassette can be inserted into the E1 region, yielding a recombinant E1/E3-deleted Ad vector. In one of the most frequent systems to produce these RAds the viral genomes are transfected into human 293 cells that express the E1 proteins *in trans* allowing for E1-deleted Ad vector replication and packaging. However,

these RAd vectors have residual expression of viral genes that leads to a strong host immune response, resulting in the generation of a high titer of neutralizing anti-capsid antibodies that inhibit re-infection with the same serotype of Ad vector. At high viral doses, this viral gene expression leads to immune-mediated cellular cytotoxicity, which results in an immune-mediated loss of the Ad vector-transduced cells. To overcome this limitation, a series of Ad vectors with multiple deletions has been developed, eliciting reduced toxicity in animal models. More recently, a newer generation of helper-dependent high-capacity adenoviral vectors (also known as high-capacity, "gutless" or "guttled" vectors), which are devoid of all viral coding sequences have been developed.

Adeno-associated virus vectors

The adeno-associated virus⁴ is a linear, single-stranded DNA parvovirus, which is currently being developed as a gene therapy vector for the treatment of numerous diseases. During AAV recombinant vector (rAAV) production, *cap* and *rep* sequences are provided by a helper plasmid, and a recombinant vector is easily rescued by co-infection with adenovirus.

Retroviral and lentiviral vectors

Retrovirus- and lentivirus-derived vectors constitute a group of RNA viral vectors that can integrate into the host cellular genome unlike many other viruses that remain episomal. This ability to integrate into host DNA makes these vectors an attractive choice for gene therapy applications. Once the vector integrates, the transgene of interest will be copied during DNA replication of the host cell, allowing for prolonged (up to 2 years) transgene expression, which is essential for chronic therapeutic applications. However, there have been documented cases in which transgene expression was gradually silenced over time.

Herpes simplex type 1 vectors⁶

Herpes simplex virus type 1 is a human pathogen and has probably infected around 80% of the population. Once infection occurs in the epithelium, the virus travels to the CNS ganglia via retrograde axonal transport, where it establishes latency as an episome; under reactivation conditions, the virus spreads from the ganglia via anterograde transport to the epithelium (recurrence) where virus replication is active. HSV establishes latency in the nervous system and can therefore exist in an individual for his or her entire lifetime. HSV contains a double-stranded DNA molecule with two unique sequences, which are linked by internal repeat sequences and flanked by terminal repeats. There are about 80 genes in the viral genome, half of which are nonessential. These nonessential genes are deleted during vector development allowing for approximately 50 kb of foreign DNA to be inserted. HSV vectors are rendered recombination deficient to ensure that the stringent biosafety requirements necessary for gene transfer and gene therapy applications are met. Recombination



deficiency is achieved by deleting the immediate early genes, such as ICP0, ICP4, ICP22, ICP27, and ICP 47, which are needed for lytic infection and expression of all other viral proteins. The ICP0 is needed for viral replication and is also essential for long-term, high-level transgene expression. Therefore, the production of multiple immediate early gene-deleted HSV-1 vectors is a balance between efficiency, persistent transgene expression, and cytotoxicity. Mutations in the Vmw65 and IE3 genes are also used to prevent viral replication. Some viral gene deletions can lead to cytotoxicity in the brain. The use of multiple deletions, including Vmw65, IE1, and IE3, results in reduced cytotoxicity and is therefore a promising vector manipulation for gene therapy.

Non-viral vector-mediated gene delivery

In trying to circumvent safety issues inherent to the use of viral vectors, development of numerous nonviral vectors is actively being pursued. The underlying principle of non-viral vector systems is to complex DNA that carries a therapeutic gene with molecules that will facilitate DNA entry into the cells of interest. Complexed DNA binds to the cell membrane, triggering either nonspecific or receptor-mediated endocytosis. Upon entry into the cell these complexes are contained in endosomes. The ability of these complexes to escape from endosomes before lysosomal enzymes destroy them is an essential characteristic of a successful non-viral vector. Once released from the endosomes, these complexes must enter the nucleus to undergo transcription. To be successfully transcribed, complexed DNA must be released from its carrier molecules and stably express RNA. Aside from avoiding the issues of viral safety, nonviral vector DNA remains episomal, allowing long-term and also high levels of gene expression, i.e., in muscle cells for Duchenne muscle dystrophy, glial cells and primary neurons, fibroblasts for lysosomal storage disorders, glial cells for cerebral ischemic diseases, and glioblastoma cells. There are several types of non-viral vector systems being explored to find optimal carrier systems. Although uncomplexed DNA has been successfully used to transfect skeletal muscle, systemic administration has been unsuccessful due to clearance of DNA by serum nucleases.

Regulatable gene expression systems for gene therapy applications⁷

Gene therapy's success hinges on several factors including site, duration, and levels of gene expression. Regulatory systems have been developed to control the temporal expression of a target gene *in vitro* and *in vivo*. Currently, the tetracycline regulatory system is the most widely used and versatile system.

CONCLUSION

Regulatable gene expression systems are an attractive gene therapy development, and potential applications have been assessed in a wide variety of preclinical laboratory models of disease. In this article, most commonly studied diseases, namely cancer, diabetes, arthritis, and ischemia, which make use of inducible gene expression systems for the treatment in pediatrics.

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