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Review

Gene therapy: A review article

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The introduction of nucleic acids into cells has as a purpose of medical condition or disease. Currently, gene therapy studies a broad range of potential therapeutic interventions, including the body's immune reaction to tumors, new blood vessels in the heart to alleviate heart attacks and to stop HIV-replication in patients with AIDS (Coleman et al., 2003). There is also renewed emphasis on the gene therapy of genetic diseases, such as hemophilia A and B, and cystic fibrosis. Human gene therapy experimentation raises many issues. In this review article, background of gene therapy, introduction, genetic diseases, gene function, germ line gene therapy, hurdles in gene therapy, methods for gene therapy, *ex vivo, in vitro* and *in vivo*-gene therapy, risks associated with gene therapy, have been given.

Key words: Gene therapy, vectors, genetic diseases, methods for gene therapy.

INTRODUCTION

Advances in the molecular biology have been made early in the 1980. It has been already studied that human genes can be sequenced and cloned. Scientists search new methods for easily producing of proteins, such as insulin in diabetic patients. Modified bacteria, introduced in the body, can be harvested and injected in people, who cannot produce it naturally. Scientists try to introduce genes straight into human cells, focusing on diseases, caused by single-gene defects, such as cystic fibrosis, hemophilia, muscular dystrophy and sickle-cell anemia (Kay et al., 2000). Gene therapy for hemophilia B and other hereditary plasma protein deficiencies have shown great promise in pre-clinical and early clinical trials (Mannucci et al., 2001).

Gene therapy can be broadly defined as a transfer of genetic material to cure a disease or at least to improve the clinical status of a patient. One of the basic concepts of gene therapy is to transform viruses into genetic shuttles, which would deliver the gene of interest into the target cells. Based on the nature of the viral genome, these gene therapy vectors could be divided into RNA and/or DNA viral vectors. The majority of RNA virus-based vectors have been derived from simple retroviruses like murine leukemia virus. A major shortcoming of these vectors is that they are not able to transduce non-dividing cells. This problem may be overcome by use of novel retroviral vectors, derived from lentiviruses, such as human immunodeficiency virus (HIV) (Ehmann et al., 1994). The most commonly used DNA virus vectors are based on adenoviruses and adeno-associated viruses (AAVs). Although, the available vector systems are able to deliver genes *in vivo* into cells, the ideal delivery vehicle has not been found. Thus, the present viral vectors should be used only with great caution in human beings and further progress in vector development is necessary. Gene transfer technologies are promising tools to manipulate donor T-cell immunity to enforce graft-versus-tumor/graft-versus-infection, while prevention or control of graft versus host disease. For this purpose, several cell and gene transfer approaches have been investigated at the pre-clinical level and implemented in clinical trials (Mastaglio, 2010). The nuclear envelope represents a key barrier to successful

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non-viral transfection and gene therapy both *in vitro* and *in vivo*. Although the main purpose of the nuclear envelope is to partite the cell to maintain cytoplasmic components in the cytoplasm and nuclear components, the most notably genomic DNA in the nucleus, this function poses a problem for transfections, in which exogenous DNA is delivered into the cytoplasm. After delivery to the cytoplasm, nucleic acids rapidly become more complex, with cellular proteins that mediate interactions with the cell machinery for their traffick. Thus, these proteins are that, in essence, which control the nuclear import of DNA, and we must also understand their activities in cells (Lam, 2010). Gene therapy for neurological, and in particular, neurodegenerative, diseases, is now a reality. A number of early phase clinical trials have been completed and several are currently in progress. In view of this, it is critically important to be evaluated the immunological risk, associated with neurological gene therapy, which has clear implications for trial safety and efficacy. Moreover, it is imperative in particular to identify factors, indicating potential high risk (McMenamin, 2010). Viral vectors are potent gene-delivery platforms, used for the treatment of genetic and acquired diseases. However, just as viruses have evolved to infect cells efficiently, the immune system has evolved to fight off what it perceives as invading pathogens. Therefore, innate immunity and antigen-specific adaptive immune responses against vector-derived antigens reduce the efficacy and stability of *in vivo*-gene transfer. In addition, a number of vectors are derived from parent viruses that humans encounter through natural infection, resulting in pre-existing antibodies and possibly in memory responses against vector antigens. Similarly, antibody and T-cell responses might be directed against therapeutic gene products that often differ of the endogenous non-functional or absent protein that is being replaced. As details and mechanisms of such immune reactions are uncovered, novel strategies are being developed, and vectors are being specifically engineered to avoid, suppress and/or manipulate the response, ideally resulting in sustained expression and immune tolerance to the transgene product (Nayak, 2010).

Haematopoietic stem cell transplantation (HSCT) is now widely used for treatment of primary immunodeficiencies (PID). For patients with specific disorders (severe combined immunodeficiency (SCID)-X1, adenosine deaminase deficiency (ADA)–SCID, X-chronic granulomatous disease (CGD) and Wiskott–Aldrich Syndrome (WAS), who lack a suitable human leukocyte antigen- (HLA)-matched donor, gene therapy has offered an important alternative treatment option (Qasim, 2009). Artificial chromosomes (ACs) are highly promising vectors for use in gene therapy applications (Isman et al., 2008). They are able to maintain expression of genomic-sized exogenous transgenes within target cells, without integrating into the host genome. Although, these vectors have huge potential and benefits, in comparison with normal expression constructs, they are highly complex, technically challenging to construct and difficult to deliver to target cells (Macnab, 2009). In the last two decades, remarkable advances have been made in the development of technologies used to engineer new aptamers and ribozymes. This has encouraged interest among researchers, who seek to create new types of gene-control systems that could be made to respond specifically to small-molecule signals. Validation of the fact that RNA-molecules can exhibit the characteristics, needed to serve as precision genetic switches, has come from the discovery of numerous classes of natural ligand-sensing RNAs, called ribo-switches. Although a great deal of progress has been made toward engineering useful designer ribo-switches, considerable advances are needed before the performance characteristics of these RNAs match those of protein systems that have been co-opted to regulate gene expression (Breaker, 2009). Pulmonary gene therapy cures diseases such as cystic fibrosis, α1-antitrypsin deficiency, lung cancer and pulmonary hypertension. Efficient expression of delivered genes in target cell types is essential for the achievement of this goal (Sinn, 2009).

GENETIC DISEASES

Cystic fibrosis, blood disorders, muscular dystrophy and diabetes.

Understanding gene function

From the estimated 30 to 50,000 genes, we know the function of a very few. Attempting gene therapy how every one of them works could address only some of the genes, implicated in particular diseases. Likewise, genes may have more than one function.

Germ line gene therapy

This technique involves the genetic modification of germ cells. Such therapy would change the genetic make up of the egg or sperm of an individual and would be carried on to future generations. This would offer the possibility of removing an inherited disorder from a family line forever.

Hurdles in gene therapy

The therapeutic genes are inserted into the body through specific constructs, called vectors, which deliver therapeutic genes to the patients' cells. The most common vectors are viruses. Scientists try to manipulate

the viral genome to remove the disease-causing genes and introduce therapeutic genes. The introduction of viruses in the body might cause side effects like toxicity, immune and inflammatory responses, as well as gene control and targeting issues.

METHODS FOR GENE THERAPY OF CANCER

Viruses
Naked DNA (vector-free)
Liposomes
Protein-DNA complexes
Gene gun
Calcium phosphate precipitation
Electroporation
Intracellular microinjection

Administration

Ex vivo

Cells are removed, genetically modified and transplanted back into a patient.

In vivo

Direct transfer of genetic material into patient.

Choices of vectors

Viral vectors:
Retrovirus
Adenovirus
Adeno-associated virus
Herpes Simplex Virus

Non-viral vectors

Liposome
DNA–polymer conjugates
Naked DNA

Gene therapy step by step

Bone marrow from the patient is removed and grown in laboratory conditions (Grunebaum et al., 2006). The cells in culture are exposed to the virus, carrying the desired gene. After infection and integration of the desired gene in the cells' DNA, the cells are returned in the patient by injection into a vein. This technique is called *ex vivo*, because the gene is transferred to the cells, while they are outside the patient's body. In the *in vivo*-technique, the gene of interest is transferred to cells inside the patient's body by using of liposomes (fatty particles).

Risks associated with current gene therapy

Viruses can infect more than one cell types. Viral vectors might alter more than the intended cells. With other words, the external gene might be inserted into the wrong location in the DNA, causing cancer or other damage. When DNA is injected directly into a tumor, there is a risk some DNA to be introduced into germ cells, producing inheritable changes. The gene might be over-expressed (toxicity); the viral vector could cause inflammation or immune reaction; the virus could be transmitted to other individuals or the environment.

RESEARCH STUDY

Restorative effect of insulin-like growth factor-I gene therapy in the hypothalamus of senile rats with dopaminergic dysfunction

Insulin-like growth factor-I (IGF-I) is a powerful neuroprotective molecule that is strongly induced in the central nervous system after different insults. In one study a recombinant adenoviral vector (RAd-IGFI) harboring the gene for rat IGF-I was constructed and used to implement IGF-I gene therapy in the hypothalamus of senile female rats, which display hypothalamic dopaminergic (DA) neurodegeneration and as a consequence, chronic hyperprolactinemia. Restorative IGF-I gene therapy was implemented in young (5 months) and senile (28 months) female rats, which received a single intrahypothalamic injection of 3 x 10(9) plaque-forming units of RAd-betagal (a control adenoviral vector expressing beta-galactosidase) or RAd-IGFI and were killed 17 days post-injection. In the young animals, neither vector modified serum prolactin levels, but in the RAd-IGFI-injected senile rats a nearly full reversion of their hyperprolactinemic status was recorded. Morphometric analysis revealed a significant increase in the total number of tyrosine hydroxylase-positive cells in the hypothalamus of experimental as compared with control senile animals (5874±486 and 3390±498, respectively). It was concluded that IGF-I gene therapy in senile female rats is highly effective for restoring their hypothalamic DA dysfunction and thus reversing their chronic hyperprolactinemia (Hereñú et al., 2007).

The ependimal route for Insuline-Like Growth Factor-1, Gene Therapy in the brain.

In one study, it has been shown that intracerebroventricular administration of the peptide

insulin-like growth factor-1 (IGF-1) is effective neuroprotective strategy in the brain of different animal models, a major advantage being the achievement of high concentrations of IGF-1 in the brain without altering serum levels of the peptide. In this study high performance recombinant adenoviral (RAd) vectors expressing their transgene were used under the control of the potent mouse cytomegalovirus immediate early (mCMV) promoter, to transduce brain ependymal cells with high efficiency and to achieve effective release of transgenic IGF-1 into the cerebrospinal fluid (CSF). RAd vectors expressing either the chimeric protein (TK/GFP) fus (green fluorescent protein fused to HSV1 thymidine kinase) or the cDNA encoding rat IGF-1, both driven by the mCMV promoter were constructed. The vectors were injected into the lateral ventricles of young rats and chimeric GFP expression in brain sections was assessed by fluorescence microscopy. The ependymal cell marker vimentin was detected by immunofluorescence and nuclei were labeled with the DNA dye DAPI. Blood and CSF samples were drawn at different times post vector injection. In all cerebral ventricles, vimentin immunoreactive cells of the ependyma were predominantly transduced by RAd-(TK/GFP)fus, showing nuclear and cytoplasmic expression of the transgene. For tanycytes (TK/GFP)fus expression was evident in their cytoplasmic processes as they penetrated deep into the hypothalamic parenchyma. Intracerebroventricular injection of RAd-IGF-1 induced high levels of IGF-1 in the CSF but not in serum. It was concluded that the ependymal route constitutes an effective approach for implementing experimental IGF-1 gene therapy in the brain (Hereñúb et al., 2009).

Protection and repair of the nigrostriatal dopaminergic system by GDNF *in vivo*

Glial cell line derived neurotrophic factor (GDNF), a recently cloned new member of the transforming growth factor-β superfamily, promotes survival of cultured fetal mesencephalic dopamine neurons and is expressed in the developing striatum. Dopaminergic neurotoxin l–methyl–4–phenyl–1,2,3,6–tetrahydropyridine (MPTP) produces parkinsonian symptoms in man and GDNF exert protective or regenerative effects *in vivo* in the adult nigrostriatal dopamine system in C57/B1 mice. GDNF injected over the substan-tia nigra or in striatum before MPTP potently protects the dopamine system, as shown by numbers of mesencephalic dopamine nerve cell bodies, dopamine nerve terminal densities and dopamine levels.

When GDNF is given after MPTP, dopamine levels and fibre densities are significantly restored. In both cases, motor behaviour is increased above normal levels. It was conclude that intracerebral GDNF administration exerts both protective and reparative effects on the nigrostriatal dopamine system, which may have implications for the development of new treatment strategies for Parkinson's disease (Tomac et al., 1995).

Glial cell line-derived neurotrophic factor supports survival of injured midbrain dopaminergic neurons

Glial cell-lined derived neurotrophic factor (GDNF) has been shown to promote survival of developing mesencephalic dopaminergic neurons *in vitro*. GDNF has a positive effect on injured adult midbrain dopaminergic neurons *in situ*, in a study single dose of GDNF was injected into the substantia nigra following a unilateral lesion of the nigrostriatal system. Rats were unilaterally lesioned by a single stereotaxic injection of 6-hydroxydopamine (6-OHDA; 9 µg/4 µl normal saline with 0.02% ascorbate) into the medial forebrain bundle and tested weekly for apomorphine-induced (0.05 mg/kg s. c.) contralateral rotation behavior, Rats that manifested >300 turns/hour received a nigral injection of 100 µg GDNF, or cytochrome C as a control, 4 weeks following the 6-OHDA lesion, Rotation behavior was quantified weekly for 5 weeks after GDNF. Rats were subsequently anesthetized, transcardially perfused, and processed for tyrosine hydroxylase immunohistochemistry. It was found that 100 µg GDNF decreased apomorphine-induced rotational behavior by more than 85%. Immunohistochemical studies revealed that tyrosine hydroxylase immunoreactivity was equally reduced in the striatum ipsilateral to the lesion in both cytochrome C and GDNF-injected animals. In contrast, large increments in tyrosine hydroxylase immunoreactivity were observed in the substantia nigra of animals treated with 100 µg of GDNF, with a significant increase in numbers of tyrosine hydroxylase-immunoreactive cell bodies and neurites as well as a small increase in the cell body area of these neurons. It was concluded that GDNF can maintain the dopaminergic neuronal phenotype in a number of nigral neurons following a unilateral nigrostriatal lesion in the rat (Bowenkamp et al., 1995).

Dopaminergic neurons protected from degeneration by GDNF gene therapy

Glial cell line-derived neurotrophic factor (GDNF) supports growth and survival of dopaminergic (DA) neurons. A replication-defective adenoviral (Ad) vector encoding human GDNF injected near the rat substantia nigra was found to protect DA neurons from the progressive degeneration induced by the neurotoxin 6-hydroxydopamine (6-OHDA) injected into the striatum. Ad GDNF gene therapy reduced loss of DA neurons approximately threefold 6 weeks after 6-OHDA lesion, as compared with no treatment or injection of Ad lacZ or Ad mGDNF (encoding a biologically inactive deletion mutant GDNF). It was concluded that Ad vector-mediated GDNF gene therapy may slow the DA neuronal cell loss in

humans with Parkinson's disease (Choi-Lundberg et al., 1997)

DISCUSSION

Plasmid DNA (pDNA) expression vectors are fundamental to all forms of non-viral gene transfer. Principles of pDNA design and production include the impact of bacterially-derived sequences on transgene expression and minicircle approaches to minimize their effects.

The impact of inclusion of DNA-elements such as scaffold matrix attachment regions (S/MARs), transcription factor (TF)-binding sites and tissue-specific promoters are described. The benefits of eliminating CG-dinucleotides (CpGs) from the pDNA are also considered (Gill, 2009). In the past 2 years, new gene-targeting approaches, using adeno-associated virus (AAV) and designer zinc-finger nucleases, have been successfully applied for production of genetically-modified ferrets, pigs, mice and zebrafish. Gene targeting, using these tools, has been combined with somatic cell nuclear transfer and germ cell transplantation to generate gene-targeted animal models. These new technical advances, which do not require the generation of embryonic stem cell-derived chimeras, would greatly accelerate the production of non-mouse animal models for biomedical research (Yan, 2009). In the last two years, significant advances in understanding of the cellular-innate responses, elicited or activated by the entry of amplicon particles, have been seen, which might, in part, explain the transient nature of the often observed in infected with helper-free amplicon stocks cells transgene expression. At the technological level, the most consistent progress has been in strategies to enhance the stability of transgene cassettes, either through integration into host chromosomes or through the conversion of the amplicon genome into a replication-competent extra chromosomal element (Epstein, 2009). Gene therapies, aiming the treatment of arthritis and tissue repair, continue to be the most active areas of research for bone and joint diseases. In the past 2 years, two trials in rheumatoid arthritis have been completed: A phase I study, reporting safety and a phase I/II study, and that has not yet be published. An additional, small study has reported the first evidence of clinical efficacy.

Two phase I trials of gene therapy for osteoarthritis have also been initiated. There is much pre-clinical activity in developing AAV vectors for future trials in the gene therapy of arthritis. The research onto the tissue repair and regeneration remains at a pre-clinical stage, but a considerable volume of research attests to the promise of gene transfer in this arena, especially in the context of bone healing, is necessary. For tissue repair, the major research questions are still which genes should be used and how they could be best delivered (Evans, 2009).

CONCLUSION

In the area of gene therapy, it is clear that many exciting innovations are emerging. While many of these new gene-therapy and biotech products might yet have unknown risks, they also have the potential for tremendous patient benefit. In this review article, several cellular and gene transfer approaches have been investigated at a pre-clinical level, and some clinical trials have been made. A number of gene-therapy trials have been completed and several of them are currently in progress. Viral vectors are potent gene-delivery platforms, used for treatment of genetic and acquired diseases.

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