INTRODUCTION

Gene Therapy

It is the replacement of person’s faulty genetic material with normal genetic material to treat or cure a disease or abnormal medical condition (US Food and Drug administration).

VECTORS FOR GENE THERAPY

Vectors is defined as a vehicle to deliver the gene of interest. There are mainly two types of vectors:

1. Viral vectors
2. Nonviral vectors.

1. Viral Vectors include adenovirus, adeno-associated virus, retrovirus and herpes simplex virus.
   a. Adenoviruses: They infect both dividing cells and nondividing cells. Adenoviruses do not integrate the foreign DNA into host cell’s rather the foreign DNA exists independently in the nucleus (so called episome). Adeno-associated virus, the smallest of three vectors listed, can accommodate only about half as much as foreign DNA as the others.
   b. Retroviruses: Retroviruses infect only dividing cells. They integrate the foreign DNA into the host cell chromosomes and thus lead to stable expression. However, the gene insertion is not controlled, and it occurs in such a way as to cause a mutation of the cell.

2. Nonviral Vectors can further classified into physical and chemical vectors.
   Physical vectors include electrophoration, microinjection, use of Ballistic particles.
   Chemical vectors include calcium vectors, lipids, protein complexes.
   a. Electrophoration: In this method, electrical current creates transient holes in the cell membrane through which DNA can be transferred.

b. Microinjection: In this method, DNA is introduced in a single cell.

c. Use of Ballistic Particles: The plasmid DNA is coated onto tungsten or gold particles. Accelerated force is generated by high-voltage electronic spark, or helium discharge to propel the beads into the tissue.

d. Calcium Vector: The ultra-low size, highly mono-dispersed DNA doped calcium phosphate nanoparticles protect from the external DNAse environment can be used safely to transfer the encapsulated DNA under in vitro and in vivo condition.

e. Lipid Vectors: They are produced by a combination of plasmid DNA and a solution that results in the formation of liposome. This fuses with the cell membrane of a variety of cell types, introducing plasmid DNA into the cytoplasm and where it is transiently expressed.

f. Protein Complex: Several groups developed cell-specific DNA delivery systems that utilize unique cell surface receptor on the target cell. By attaching the ligands recognized by such a receptor to the transfer DNA, the DNA ligands complex become selectively bound and internalized into the target cell.

APPLICATION IN DENTISTRY

Bone Repair

An area of real clinical importance to dentistry, with considerable research progress, uses gene therapy to repair bony lesions. Studies by researchers at the university of Michigan School of Dentistry have used ex vivo methods to transfer genes encoding bone morphogenetic proteins, or BMPs. BMPs are agents well established in induction of both orthotopic and ectopic bone formation. In ex vivo studies, researchers accomplish the actual gene transfer in a tissue culture environment and then place the transduced cells carrying the foreign genes back into the host.

ABSTRACT

As evolution is a continuous process with the advancement of the technology, the researches are in continuous to understand the cellular and molecular basic of every disease. As in most of the diseases conventional method is not giving satisfactory results, thus focus is on gene therapy located to treat not only inherited disease but also acquired ones. The gene therapy has broad spectrum application in the field of dentistry like in cancerous and precancerous condition, salivary gland disorders, autoimmune diseases, bone repair, DNA vaccination, etc. Thus, aim of this article is to focus in brief of the basic of gene therapy and about its applications in the field of dentistry.

Keywords: Gene, Vector.
animal models, the Michigan research group has shown that several different cell types, such as nonosteogenic fibroblasts (from human gingiva and dental pulp) and myoblasts, as well as osteoblasts can express the BMP-7 gene after being infected with an adenoviral vector. Then these cells are able to differentiate into bone-forming cells when placed in an osseous defect in vivo.

Other studies, conducted by researchers at the Hebrew university-Hadassah have used mesenchymal stem cell-mediated gene therapy for bone regeneration. Genetically engineered mesenchymal stem cells expressing BMP-2 induced increased formation of new blood vessels as well as new bone. These studies also showed that the genetically engineered stem cells were able to engraft, differentiate and display regulatory behaviors. Investigation demonstrated by Alden and colleagues showed that it is possible to directly deliver the BMP-2 gene in vivo to tissue via an adenoviral vector (vs using ex vivo cellular re-engineering) and thus achieve healing of mandibular osseous defects.

This general strategy tries to enhance a natural reparative response by supplementing the regenerative site with therapeutic proteins. It is clearly possible to manipulate a variety of cell types by different methods to express BMP genes and, thereafter, for these transduced cells to mediate bone regeneration. Additionally, it is likely that other genes soon will be available to facilitate localized regeneration of bone for periodontal and oral surgical applications. For example, a novel strategy, recently reported by another group at the University of Michigan, involved transfer of the platelet-derived growth factor gene to periodontal cells and resulted in DNA synthesis and cellular proliferation.

Gene transfer to salivary glands: Salivary glands are excellent target sites for gene transfer. They are capable of producing large amounts of proteins, and are a site where gene transfer can be readily accomplished in a minimally invasive manner (by means of intraductal cannulation). Human salivary glands are also encapsulated, a circumstance likely to minimize the undesirable access of administered vectors and transgenes to other tissues.

Main goal in developing gene transfer with salivary glands was to provide novel and effective therapies for patients, who suffer from irreversible salivary gland dysfunction resulting from either irradiation for head and neck cancers or the autoimmune damage occurring with Sjögren’s syndrome. Later two additional clinical goals for salivary gland gene transfer, both involving the use of genes as pharmaceuticals (gene therapeutics). Salivary glands are by nature a secretory tissue and certainly a logical site for local (oral, pharyngeal and esophageal) applications of gene therapeutics requiring the exocrine secretion of transgene products in saliva. Additionally, salivary glands could be used for gene therapeutic applications with systemic single-protein deficiency disorders.

Compared with salivary glands, other tissue sites have both advantages and disadvantages for systemic gene therapeutics. However, it is unlikely that any single tissue is ideal for all possible uses, and salivary glands may be useful for some specific purposes.

A variety of genes have been transferred including genes encoding hormones (growth hormone, insulin), an antimicrobial agent (histatin 3, or H3), membrane proteins (aquaporin-1 and aquaporin-5), a transcription factor (E2F-1), protease inhibitors (α1-antitrypsin and kallikrein), a protein affecting apoptosis (Fas ligand) and several nonmammalian “reporter proteins” (β-galactosidase, chloramphenicol transferase and luciferase). For repair of damaged salivary glands, initial approach was to insert a gene encoding a water channel protein, aquaporin-1, or AQ1, into radiation-surviving (primarily ductal) salivary cells to convert these nonsecretory cells into a secretory phenotype. An adenovirus-encoding human AQ1, termed “AdhAQ1,” was administered to hypofunctional rat submandibular glands that had been irradiated four months earlier with a dose of 21 gray. Three days after gene transfer, the treated glands were secreting saliva at flow rates indistinguishable from those of nonirradiated control glands.

After these encouraging results, researchers tested safety and efficacy of AdhAQ1 in rhesus monkeys. At 20 weeks after irradiation, the animals received either AdhAQ1 or a control virus. The animals tolerated the single doses of AdhAQ1 well, but the salivary results were inconsistent. AdhAQ1 enhanced salivary secretion only modestly in some animals. They are not sure why the results were not as encouraging in the monkey model as in the rat model. One possible technical explanation is that in the monkey, the glands were underfilled by the vector/infusate volume used. A subsequent study in mice showed that maximal transgene expression occurred when glands were somewhat overfilled. Thus, additional animal studies are continued to decide if it is useful to pursue the AQ1 gene transfer strategy clinically.

The second clinical goal was to use gene transfer to deliver a gene product locally to treat disorders of the mouth and upper gastrointestinal tract. The clinical condition, we addressed initially, was azole-resistant candidiasis in immunosuppressed patients. The third clinical goal for gene transfer to salivary glands was to correct systemic single-protein disorders.

Autoimmune disease and gene transfer: Sjögren’s syndrome, or SS, is an autoimmune disease that leads to the destruction of salivary gland tissue and marked reductions in salivary flow. Recently, the gene transfer application of immune modulation appears to have potential for treatment of autoimmune diseases. There are no effective conventional therapies for SS and other autoimmune disorders. Most autoimmune diseases are complex and unlikely will be corrected by the reconstitution of a single missing gene. Although a successful gene transfer strategy for SS has yet to be experimentally proven, there has been considerable progress toward this goal and for other, related autoimmune diseases.

The strategy, which researchers are using, is that biological factors that enhance Th2 functions and suppress Th1 cells likely will be efficacious for therapy. One positive reason to consider immunomodulation of salivary glands in patients with SS using gene transfer is that the intervention uses a targeted, local delivery with selective tissue expression. However, a major concern about using gene transfer vectors with patients, who have autoimmune disease is the possibility of an immunological reaction to the vector. In response to this concern, many investigators developing gene transfer-based treatments for autoimmune diseases, including SS, now are using recombinant adeno-associated virus, or AAV, serotype 2, as the vector of choice. AAV vectors are much less immunogenic than adenoviral vectors.
Initial studies have focused on transferring the gene for human, or h, IL-10 using a recombinant AAV2 vector. IL-10 has a broad spectrum of biological effects. Among these are the inhibition of antigen-specific T-cell proliferation, of cytokine production by Th1-like cells and of macrophage-dependent antigen presentation. Conversely, it seems unlikely that overexpression of hIL-10 will cause severe disturbances in a host’s protective immune responses. hIL-10 immunomodulatory therapy has been tried and shown to be useful in preclinical models of other autoimmune diseases, including rheumatoid arthritis.

**Pain:** Managing or eliminating pain is a major part of dental practice. The use of gene transfer technology offers a potentially novel approach to manipulate specific, localized biochemical pathways involved in pain generation. Gene transfer may be particularly useful for managing chronic and intractable pain. Several studies in animal models have shown that viral mediated transfer of genes encoding opiate peptides to peripheral and central neurons can lead to antinociceptive effects. While considerably more research is needed before gene transfer can be tested clinically as a strategy for chronic pain management.

**DNA vaccinations:** For many years, dental scientists have tried to use classical vaccination technology to eradicate dental caries or periodontal diseases, thus far achieving mixed success. In the last decade, gene transfer research has led to a novel way to achieve vaccination: Directly delivering DNA in a plasmid vs the traditional administration of a purified protein or an attenuated microbe. This prediction was demonstrated in an animal study published in 1999 by Kawabata and colleagues, of Osaka University’s Faculty of Dentistry in Japan. They achieved a targeted salivary gland immunization using plasmid DNA encoding the *Porphyromonas gingivalis* fimbral gene. This gene led to the production of fimbrial protein locally in the salivary gland tissue of mice, with the consequent production of specific salivary immunoglobulin A, or IgA, and immunoglobulin G, or IgG, antibodies and serum IgG antibodies. Additionally, they observed the generation of antigen-specific cytotoxic T lymphocytes in immunized mouse spleen cells. Thus, one might expect that the secretory IgA secreted in saliva could neutralize *P. gingivalis* and limit, its ability to participate in plaque formation. Furthermore, any secreted fimbrial protein in saliva could bind to pellicle components and also inhibit the attachment of *P. gingivalis* to the developing plaque. Although, applications of DNA vaccination are in the earliest stages of use with oropharyngeal tissues, it seems reasonable to suggest that these approaches will play a role in future strategies for preventing periodontal diseases and dental caries.

**Gene transfer to keratinocytes:** This kind of transfer has focused on the technology of reengineering keratinocytes ex vivo using retrovirus. The main aim is to transfer foreign genes into mucosal and epidermal keratinocytes for normalization of tissue structure and epidermal function. Keratinocytes are very favorable in these sites as:

- The area is easily accessible so monitoring is adequate.
- Preclinical assessment is accurate since culture models are established.
- Expression of therapeutic genes can be achieved with the use of topically applied agents.

- Procedures for transplanting keratinocytes sheets already established because of their application for burn patients.
- It is reversible because genetically modified tissue can be excised.

**For Head and Neck Cancer:** The prognosis of patients with squamous cell carcinoma of the head and neck (SCCHN) is poor. The incidence of this cancer has been gradually increasing over the past 20 years and it is now the fifth leading cause of cancer incidence and the sixth leading cause of cancer-related death in the world.

**Advexin (INGN 201, Ad5CMV-p53)** Ad vexin (INGN 201, Ad5CMV-p53; Introgen Therapeutics, Inc.) is an adenovirus (type 5) in which the E1 region is replaced with the cDNA of the p53 gene and is driven by a cytomegalovirus (CMV) promoter. The p53 gene is located on chromosome 17p in humans and it encodes a 39 kDa amino acid protein that is critical to tumor biology. Inactivation of p53 singling pathway can allow proliferation of damaged cells and results in tumor formation. Delivery of the wild type p53 gene to a cancer cell via a modified adenoviral vector induces expression of wild-type p53 protein and triggers growth arrest or apoptosis, causing tumor growth inhibition.

**Gencidine** Gencidine is another different molecular entity that combines an adenovirus type 5 vector with a p53 expression cassette, using the RSV’ promoter and BGH (A) tail.

In phase I trial using the adenoviral vector SCH-58500, 16 patients with HNSCC received escalated doses ranging from 7.5 × 10^6 PFU to 7.5 × 10^12 PFU. Toxicity was limited to grade 1 to 2 fever and injection pain. One patient achieved a partial remission (PR), which correlated with the induction of apoptosis and transgene expression.

Recently, the first randomized clinical trial of p53 gene therapy was reported. Ninety patients with SCCHN were randomly allocated to receive either intratumoral injections of Ad-p53 in combination with radiation therapy (70 Gy/8 weeks) or radiation therapy alone. Complete remission was seen in 64.7% of patients receiving Ad-p53 combined with radiation therapy compared with 20% of patients receiving radiation therapy alone, which was statistically highly significant. This clinical trial formed the basis for approval in head and neck cancer of Ad-p53 by the China State Food and Drug Administration, thus making Ad-p53 the first gene therapy approved for humane use.

**Onyx (DL1520)** Onyx (DL1520) is a replication-conditional adenovirus that is defective in the early regulator protein E1B, which binds to and inactivates p53 to promote its own activation.

Cells containing an intact p53 pathway are thus predicted to inhibit replication of an E1B 55 kD-deficient virus. In contrast, p53-deficient cells, such as those of a tumor, would be expected to allow efficient viral replication and subsequent cell killing. However, several groups demonstrated that ONYX-015 efficiently replicates in many tumor cells types with wild-type p53. This
apparent contradiction was resolved through examinations of p14ARF, a tumor suppressor gene, whose product functionally stabilizes p53. Loss of p14ARF was identified as a mechanism that allows ONXY-015 replication in tumor cells retaining wild-type p53.

**H-101**

H-101 is an E1B-55k and partly E3 deleted adenoviral vector comparable to ONXY-015. In a randomized phase 3 clinical trial of H-101 along with 5-FU and cisplatin, virus particles were intratumoral injected daily for five consecutive days every 3rd week. Chemotherapy alone shows success rate about 39%, while combination of chemotherapy and H-101 shows 78% response rate. Minor side effects like fever, localized reaction at injected site were observed. On the basis of the clinical trial and results of an HLA-B7 plasmid (allovectin-7), no toxic effects were observed. Four of nine patients showed a partial response and induction of HLA-B7 expression was confirmed in two of four patients, who responded. A phase 2 trial on the basis of these data continues involving multiple injections of Allovectin-7 have been started.

**CONCLUSIONS AND FUTURE DIRECTION**

No doubt future of gene therapy is very bright. Through researchers are facing various complications like uncertainty of the response of immune system to a viral vector, lack and the response of viral vector to other cells. Despite of these, gene therapy will definitely become a potential and promising treatment modality of number of diseases especially for head and neck cancers.

Thus, there is no issue of whether but only when.

**REFERENCES**

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