Review Article

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Gene Therapy: A New Era in Dentistry

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ABSTRACT

Background: Gene therapy is the upcoming trend in medical and dental faculty which has a promising future in treatment of various diseases and ailments. It basically involves transfer of normal genes or genetic materials in a cell with defective genes in vivo or in vitro in order to cure the disease. Although researches are going on, and positive outcomes are expected and may conceivably revolutionize clinical practice in the coming years. With the help of this review, light has been thrown upon the basic concept of gene therapy, methods of gene transfer, application of gene therapy in various fields of dentistry like, bone repair, implantology, head and neck cancer, orthodontic therapy, periodontal repair etc with its pros and cons.

Keywords: Gene therapy, periodontitis, orthodontics, gene, oral cancer.

INTRODUCTION

Day by day newer techniques have been introduced in the field of medicine to provide better health options for the patients. In recent years stem cell biology has gained tremendous importance, offering future potential transplantation therapy cures for a variety of diseases. Stem cells are defined as cells that possess the ability to perpetuate themselves through self renewal and to generate mature cells of a particular tissue through differentiation. Although all the tissues do not contain stem cells.[1]

The basic concept of gene therapy is to introduce a gene from the stem cell which has the capacity to cure or prevent the progression of a disease. Gene therapy introduces a normal, functional copy of a gene into a cell which has the defective gene so that healthy progeny cells could be formed eventually to target the correction of genetic defects, eliminate cancerous cells, prevent cardiovascular diseases, block neurological disorders, and even eliminate infectious pathogens without the use of conventional drug compounds. Currently gene therapy is gaining special concern due to its role in various treatment modalities. This review article covers the basics of gene therapy along with its pros and cons and also its implication in various fields of dentistry.[2]

History

Since the earliest days of plant and animal domestication, about 10,000 years ago, humans have understood that characteristics traits of parents could be transmitted to their offspring. The first to speculate about how this process worked were ancient Greek scholars, and some of their theories remained in favor for several centuries. The scientific study of genetics began in 1850s, when Austrian monk Gregor Mendel, in a series of experiments with green peas, described the pattern of inheritance, observing that traits were inherited as separate units we know as genes. Mendel's work formed the foundation for later scientific achievements that heralded the era of modern genetics. But little was known about the physical nature of genes until 1950s, when American biochemist James Watson and British biophysicist Francis Crick developed their revolutionary model of double stranded DNA helix. Another key breakthrough came in the early 1970s, when

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researchers discovered a series of enzymes that made it possible to snip apart genes at predetermined site along a molecule of DNA and glue them back together in a reproducible manner. Those genetic advances set the stage for the emergence of genetic engineering, which has produced new drugs and antibodies and enabled scientists to contemplate gene therapy. A few years after the isolation of genes from DNA, gene therapy was discovered in 1980s.[3]

The fundamental tenets of gene therapy were led by Joshua Lederberg and Edward Tatum. [11] Historically, the discovery of recombinant DNA technology in the 1970's provided the tools to efficiently develop gene therapy. .Micheal et al. in 1977 succeeded in transferring a gene TK gene coding for thymidine kinase into mammalian cells which took a large step in the field of gene therapy. [12,13] The first approved clinical trial on gene therapy took place on September 14, 1990, on a 4 year-old girl, Ashanthi De Silva with Adenosine Deaminase (ADA)-deficiency / Severe Combined Immunodeficiency (SCID) syndrome. She was given her own T cells engineered with a retroviral vector carrying a normal ADA gene by the NIH (National Institutes of Health) team of Anderson, Blaese and Rosenberg.[14] Gene amplification, which is used in the treatment of various human diseases, was put forward by Cusack and Tanabe in 1998.[15] In 2000, the first gene therapy "success" resulted in SCID patients with a functional immune system. These trials were stopped when it was discovered that two of ten patients in one trial had developed leukemia resulting from the insertion of the gene-carrying retrovirus near an oncogene.[5]

Gene Transfer Strategies

The 3 basic elements for developing a successful gene therapy are

(1) Development of gene delivery vectors and means for their production and testing (successful and reproducible delivery and permanent incorporation into the target organ);

(2) Knowledge of molecular pathophysiology and identification of specific genes implicated in the disease; and

(3) Adaptation of laboratory animal to models of human disease.

The intersection of gene delivery vectors and molecular pathophysiology are vectors targeting specific genes. Hence, animal models are required to develop principles and protocols for in vivo gene transfer. For in vivo testing of animals, specific genes are modified in the germ line to mimic specific molecular defects, and these can serve as informative models for therapeutic gene transfer.[4]

Various methods

FUNDAMENTALS OF GENE THERAPY

There are a variety of different methods to replace or repair the genes targeted in gene therapy.[2]

- A normal gene may be inserted into a nonspecific location within the genome to replace a nonfunctional gene. This approach is most common

- An abnormal gene could be swapped for a normal gene through homologous recombination.

- The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function

- The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered

- Spindle transfer is used to replace entire mitochondria that carry defective mitochondria

Gene therapy may be classified into the following types:

Germ line gene therapy

In the case of germ line gene therapy, germ cells, i.e., sperm or eggs are modified by the introduction of functional genes, which are ordinarily integrated into their genomes. Therefore, the change due to therapy would be heritable and would be passed on to later generations.

Somatic gene therapy

In the case of somatic gene therapy, the therapeutic genes are transferred into the somatic cells of a patient. Any modifications and effects will be restricted to the individual patient only, and will not be inherited by the patient's offspring.

Gene Delivery

In general, gene therapy involves the transfer of genetic information to target cells, which enables them to synthesize a protein of interest to treat disease[5,6,7,8] The technology can be used to treat disorders that result from single point mutations.[9] The preferred strategy for gene transfer depends on the required duration of protein release and the morphology of the target site production.[10]

There are various methods of gene transfer:

1) Viral

A carrier molecule called a vector must be used to deliver the therapeutic gene to the patient's target cell. Currently, the most common vector is virus that has been genetically altered to carry a normal human DNA. As viruses have the ability to insert disease causing genes in human body, scientists are now trying to alter their property so that normal DNA can be inserted into human body.

Different types of viruses used in gene therapy

a) Retrovirus – they can create double stranded DNA copy of RNA genome.
b) Adenovirus – these are having double stranded DNA
c) Adeno associated virus – small single stranded DNA virus
d) Herpes simplex virus – double stranded DNA that has the affinity for neurons in humans.

2) Non viral

a) Direct introduction of therapeutic DNA in host cell
b) Use of artificial lipid sphere with an aqueous core. Liposome carries therapeutic DNA.

Application of gene therapy in dentistry

Gene therapy in oral cancer

Several genetic alterations have been described in the pathogenesis of oral cancer consisting of mutations of p53,p16 and p21 genes.[17] Since the protein p53 plays a role in cellcycle regulation and apoptosis, p53 gene transfer was initially tested in squamous cell carcinoma patients by injecting the primary or regional tumour with an adenoviral vector expressing wild-type p53. Adenoviral p53 (Ad-p53) was demonstrated to be safe and well-tolerated. Twenty studies on Ad-p53 as a surgical adjuvant showed good results.[18] The reconstitution of wild-type p53 function with p53-expressing adenovirus and combinational therapy using ionizing radiation and recombinant Ad-p53 has been reported to have a significant tumour-suppressant effect on various cells. The survival of SCC cell lines was inhibited after transfection with recombinant p53-expressing adenovirus.[19]

The immunologic gene therapy approach to oral cancer involves either increasing the immunogenic potential of tumour cells or augmenting the patient's immune response to a tumour. Biological molecules produced by tumour cells are found to elicit strong immune response. T-cells are the major immune cells involved in antitumour immunity.[20]

Other tumour suppressors introduced into tumour cells by gene transfer are Rb (retinoblastoma gene) and mda-7 (melanoma differentiation-associated gene-7). Their effects on apoptosis and tumour growth appear to be similar to those of interleukin-24 (Il-24), a Th1 cytokine that triggers an anti-tumour and pro-apoptotic response in the immune system.[21]Gene transfer of gene p27 was found to inhibit the cell cycle of tumour cells, inducing apoptosis and triggering the suppression of tumour growth. It has been demonstrated that gene p27 mutations are highly related to the appearance of tongue cancer. According to these results, the therapeutic use of p27 gene may in the future prove useful for the treatment of OSCC.[22]

Numerous studies have described p53 alteration as an early event in oral cavity carcinogenesis, and mutated p53 expression is frequently observed in non-cancerous epithelium adjacent to OCSS.[23] Moreover, the percentage of epithelial cells expressing mutated p53 is usually higher with greater severity of the epithelial disorder. For these reasons, one of the tumour suppressor genes most commonly used in gene therapy is the p53 gene, and

numerous viral vectors, especially adenoviral vectors, have been developed for its application.[24]

Gene Therapy for Bone Repair

Bone loss may be a consequence of several oral conditions such as periodontal disease, trauma, neoplastic pathology, reconstructive surgery or may be deficient as a result of congenital defects. Currently, efforts at addressing such bone defects revolve primarily around using substitute materials that are either synthetic or harvested. The ideal goal would be to have directed regeneration of bone to meet the necessary needs. This would require manipulation of the critical aspects of bone physiology i.e., osteoinduction, differentiation of osteoblasts and the production of osteoid matrix, osteoconduction and mechanical stimulation.[25]

This is where members of the Transformation Growth Factor (TGF) super-family such as Platelet Derived Growth Factors (PDGFs), Insulin-like Growth Factors (IGFs), Transforming Growth Factor-β (TGF-βs) and Bone Morphogenic Proteins (BMPs) have been employed for tissue engineering.

While most of these molecules act with overlapping roles, it is the group of BMPs (specifically BMP-2, BMP-4 and BMP-7) that have shown the maximum promise in their direct ability to induce bone formation, both when delivered in in vitro cultures[26] as well to local sites in vivo.[27]

Gene Therapy for Salivary Gland Disorders

Tumours and their surgical resection, autoimmune disorders such as Sjogren's syndrome, postradiation fibrosis are some conditions that can cause loss of salivary gland tissue and therapy that can restore or regenerate damaged salivary glands tissue is much desired. Salivary glandular tissue lends itself well to gene therapy since it is easily accessible via retrograde instillation of medicines through the salivary ducts and is anatomically encapsulated from adjacent structures, thereby reducing concerns associated with viral vector transduction.

An additional application of salivary gene therapy, owing to the abundant exocrine salivary protein expression is the possibility of secreting genetically engineered substances. These may be used to secrete particular substances and overcome single protein deficiencies or to deliver therapy to local areas i.e. oesophageal, pharyngeal, oral areas.[28]

Research groups using various animal models have reported salivary gland gene transfer for hormones such as growth hormone and insulin.[29,30] These studies found the expression of transgene-proteins in bloodstream, i.e., endocrine activity from the salivary glands, which may be useful to treat systemic protein deficiency disorders.

Antimicrobial and anticandidial peptides such as histatin-3 have also been transduced into salivary glands by means of cDNA, offering a novel approach to the management of candidial infections in the upper-GIT using naturally occurring antifungal proteins.[31] This has potential application since *Candida spp.* are common opportunistic infection and are a notorious cause of mucositis in HIV-compromised individuals.

Postradiation salivary hypofunction has been addressed in primates by the modulation of non secretory cells which have survived radiation into a secretory phenotype by means of an insertion of a gene, aquaporin 1 (AQP1), by means of an adenovirus vector, AdhAQP1.[32]

In Sjögren's-syndrome, the destruction of glandular tissue maybe isolated to salivary and lacrimal tissue, or may be accompanied by secondary autoimmune diseases such as rheumatoid arthritis. Due to the autoimmune nature of the disease, infiltrating CD4+ cells release proinflammatory mediators (cytokines interleutin-2, interferon-γ, tumour necrosis factor-α) as well as anti-inflammatory mediators (interleutin-4, interleutin-6, interleutin-10). The upregulation of genes coding for anti-inflammatory cytokines, therefore, has been pinpointed to offer relief for dry mouth, keratoconjunctivitis. This has been done in several studies by means of a recombinant adenovirus rAAV2hVIP, encoding the human-VIP gene (Vasoactive Intestinal Peptide).[33-35]

Gene Therapy for Chronic Pain Management

Chronic, intractable, neurologic pain within the oro facial distribution is a serious challenge to manage for the dental professional. Promising gene therapy in animal models may hold the key to solve these conditions. Researchers at the Mount Sinai School of

Medicine have engineered a virus carrying a gene for an endogenous opioid, 2 which is injected directly to the spinal fluid adjacent to the dorsal root ganglia. Since the dorsal root ganglia acts as a gateway to higher pain centres and opioids produce a morphine like blockade of this pain gateway, results lasting for up to three months have been achieved.[36] Similarly, naturally neurotropic viruses such as herpes simplex based vectors or lipid encapsulated plasmids coding for anti-inflammatory interleutin-10 have been employed to selectively inoculate neural and glial cells and inhibit allodynic neurotransmitters.[37] Researchers have also attempted direct gene therapy to the articular surface of the temporomandibular joint.[38]

Gene Therapy for Implant Osseointegration

Since alveolar bone loss is the primary reason for implant failure, and since the implant is a biologically passive structure, there is a strong interest in controlling the interaction of the bone implant interface by adjunctive means. Adenovirus-vectors encoding human Platelet Derived Growth Factor-β (PDGF-β) have been used to accelerate bone fill and improve bone density thereby improving implant osseointegration.[39] Recently, calcium phosphate nano-particles have been used as a non viral vector to transduce PDGF-gene plasmids to human fibroblasts.[40]

Gene Therapy for Orthodontic Tooth Movement

The activity of osteoclasts which are necessary for tooth remodelling during orthodontic therapy is mediated by the RANK-RANKL-OPG interaction. Here, RANKL refers to Receptor Activator of NF-kappa β-Ligand (RANKL) which is a receptor that binds to the RANK molecule and permits osteoclast precursor activation. Thus, inducing RANK-RANKL binding results in accelerated osteoclastogenesis and hastens orthodontic tooth movement by up to 150% and it may also be employed to overcome orthodontic challenges in moving ankylosed teeth.[41]

Dentin and Periodontal Regeneration

Dentinal or pulp injury secondary to carious, restorative or traumatic process is traditionally treated by means of a pulp capping procedure, which induces dentin bridge formation. Apart from calcium hydroxide, biomolecules such as porcine enamel matrix derivative (Emdogain)[42] have been made commercially available and biomolecules expressed during dentinogenesis such as Odontogenic Ameloblast Associated Protein (ODAM)[43], Amelogenin, OP-1, TGF-β, BMP-4 and BMP-2 have been shown to work experimentally by direct instillation.[44] However, a matter of concern is that several of these biomolecules favour osseous healing which may predispose to ankylotic healing of periodontal tissues.

Advantages of gene therapy[16]

• In case of 'silence' a gene. In the case of someone with

HIV, which had not yet developed into AIDS, scientists couldsave them the pain and suffering of the disease by using gene therapy to 'silence' the disease before its onset.

• Gene therapy has the potential to eliminate and prevent hereditary diseases such as cystic fibrosis and is a possible cure for heart disease, AIDS and cancer.

• These sceptics would almost certainly choose gene therapy, especially if it was the last hope for them or one oftheir loved ones – as is the case for many gene therapy patients.

Disadvantages of gene therapy[16]

• Short-lived nature of gene therapy.

• Immune response - Genes injected with a virus may trigger an immune response against the virus. Problem with viral vectors is that once inside the patient, the viral vector could recover its ability to cause disease.

• Multigene disorders - The genetic material might not get into the right cell, or the right place in the cell's DNA.

CONCLUSION

Gene therapy is the upcoming revolutionary method of treatment in field of medicine and dentistry. Although studies are still going on, the researches have proved to be successful in treatment of various ailments. This review has presented gene therapy as alternative to various treatment modalities related to dentistry which can lead to promising future.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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