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It's all in your genes! - Prospects of Gene Therapy in Dentistry.

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Abstract: *The main objective of medical and dental sciences is to understand the physiological processes occurring in humans and alleviate pain and suffering from the world. Diseases which have a multifactorial etiology are often a challenge for the clinician to treat and conventional treatment modalities do not always give satisfactory results. The missing link in these conditions is genetics, i.e. there are several disorders which are caused by alteration of genes coding for specific proteins. Gene therapy is a method by which the defective gene is repaired or replaced by a therapeutic gene with the help of suitable vectors. It has a wide array of applications from the treatment of single gene to multi-gene disorders. Salivary glands and keratinocytes are excellent target sites for gene therapy and are often used for treating systemic conditions. Its dental applications range from treatment of pain, salivary gland disorders, oral cancer, bone repair, orthodontic tooth movement, tooth regeneration and DNA vaccination for dental caries and periodontal disease. The aim of this article is to discuss the principles of gene therapy, its future prospects in the field of dentistry and the role of a dentist as a 'Gene therapist'.*

Keywords*: Gene therapy, salivary gland, oral cancer, bone repair, pain, orthodontic tooth movement, DNA vaccination, tooth regeneration*

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I. Introduction

Genes form the basic unit of heredity and genetics. They are composed of specific DNA sequences which code for specific proteins.[1] On an average, human genome constitutes of 20,000 to 23,000 genes which are responsible for controlling most of the characteristics and functions of human body. It is what makes each one of us unique. Since it controls most of the bodily functions, any alteration of genes can result in impairment of normal cell activities. Such abnormalities in cell structure and function can lead to various diseases. This forms the basis of Gene therapy, which involves insertion of functioning gene into cells, either to correct the cellular dysfunction or to provide a new cellular function. (Culver, 1994) [2] Gene therapy is carried out in two steps. First, the DNA sequence or genetic code of interest is cleaved and inserted into the vector or carrier. Thereafter, it is implanted into the target human cells to release the sequence of DNA which then integrates with the host chromosome. As it gets inserted into its designated location, it starts producing the required therapeutic proteins. [3] There are broadly two types of gene therapy- Somatic gene therapy and Germ line therapy. The former involves making alterations in the cells which are not transferred to the next generation. Whereas in the latter, changes are induced in gametes, and these changes are inheritable. Due to ethical concerns, this type of gene therapy is currently restricted to animal models. However, in the future, it can be used to treat various hereditary disorders.[3,4] The first Gene therapy trial was carried out in 1973 to treat beta-thalassemia. [5,6] In the year 1990, gene therapy was performed for the first time to treat Severe combined immunodeficiency syndrome, by inserting genes producing ADA into the WBCs. However, the results were short-lived. [6,7] Following this development, gene therapy has grown by leaps and bounds and its possible applications include treatment of various conditions like autoimmune diseases, acquired tissue damage, protein deficiencies, neoplastic diseases, to name a few. The applications of gene therapy are not just restricted to the field of medicine and it has made a substantial impact in the field of dentistry. It will eventually revolutionize the approach of treating a myriad of oral facial conditions. This article will briefly review some of the applications of gene therapy in dentistry.

II. Principles of gene therapy

Gene therapy has three basic principles.

1. Appropriate selection of gene
2. Vector selection
3. Management strategy

2.1 Selection of gene

1. Mutant gene correction: a vector is used to transfer specific DNA sequence to the target cell, which is then processed with the help of host enzymes to produce desired proteins
2. Immunotherapy: tumor-specific antigens, co-stimulatory molecules or inflammatory cytokines are introduced into the tumor cells which initiates and/or enhances the immune response against tumor cells
3. Suicide gene therapy: the gene that is transferred helps in transforming a pro-drug into a toxic metabolite leading to cell death
4. RNA interference: Micro RNA has tumor suppressor potential and can be used to modify the response to various therapeutic agents

2.2 Selection of Vector

Vector is a carrier or vehicle for the DNA sequence which is to be inserted into the target cells. They can either be viral or non-viral.

The most common viral vectors are Adenovirus, Adeno- associated virus, Retrovirus and Herpes simplex virus. They are attenuated and lack pathogenicity. However, they can induce an immune response.

Non-viral vectors include physical vectors like Naked DNA injection, synthetic vectors, virosomes, nanoparticles and biological non-viral vectors like bacteria. [5, 6,7]

1. An ideal vector should meet the following requirements
2. It should not evoke an immune response when introduced into the body
3. It should be able to protect the DNA and deliver it to the specific cell of interest
4. It should have high specificity and low toxicity
5. Longevity of expression is expected
6. Should be able to produce in large amount and should be inexpensive
7. No single vector meets all the aforementioned requirements and hence vector-selection would vary according to the clinical situation.

2.3 Management strategy

It comprises of *in vivo* and *ex vivo* gene therapy. In the former, the gene of interest is injected directly into the target cell using viral or non-viral vectors. In ex-vivo strategy, the particular cell type or its pre-cursor is collected from the subject, cultured and transduced with vector DNA and these altered cells are then reintroduced into the subject.

III. Applications In Dentistry

3.1 Salivary Gland Disorders

Salivary gland plays a very important role in maintaining the normal physiological state of the oral cavity. It has antimicrobial peptides which provide local immunity against common oral pathogens. It helps in lubrication, mastication, and digestion of food and also assists in speech. [3] Autoimmune disorders like Sjogren's syndrome, tumor resection defects and post-radiation fibrosis are some of the conditions which cause loss of functional salivary gland tissue and require restoration of gland structure and/or function. [4] Gene therapy can be used individually or in conjunction with conventional therapeutic measures to treat this condition. The first clinical trial with respect to gene therapy in salivary gland disorders was carried out in 2012.

There are reasons why Salivary gland is particularly more conducive to gene therapy than other tissues;

1. It is surrounded by a capsule which results in containment of the vectors and transgenes within the gland
2. It has the ability to secrete proteins directly into the blood stream and can hence be targeted to treat certain systemic disorders with a simple method of intraductalcannulation.
3. The position of exocrine ducts is accessible for implantation of genes. [3,8]

Ionizing radiation causes damage to the acinar cells which results in reduced salivary secretions. This can be reversed by increasing the fluid permeability pathway is introduced. Aquaporin 1 is a water channel protein which increases permeability to water and can thus reverse this detriment. Aquaporin 1 gene not only reverses radiation-induced damage, it has also been reported that this gene was successfully used to treat Sjogren's syndrome in an animal model. Recently, a clinical trial was carried out which advocate the safety of using this gene for management of xerostomia patients. However, there are a few shortcomings. It requires repeated administration to maintain its effectiveness and also may evoke a host immune response. [3]

As mentioned before, its endocrine activity can be exploited to correct several single protein disorders. For example, Growth Hormone deficiency in children can be treated by introducing human growth hormone gene into the salivary gland using recombinant adenovirus. The hormone would subsequently be released into the bloodstream by the exocrine ducts and this will result in high serum hGH. [3]\Gene coding for Histatin-3, which inhibits the growth of Candida can be transduced into the salivary gland for managing oral mucositis in HIV

patients and also treatment of upper GIT candidal infections. Similarly, antimicrobial peptides can be transduced to provide desired effects.

3.2 Bone Repair

There are several diseases and conditions which demand bone regeneration and repair. The most common conditions include trauma, periodontal disease, neoplasia, tumor resection defects, congenital diseases and autoimmune disorders. Successful Bone regeneration depends on four important elements

1. Osteoinduction
2. Differentiation of Osteoblasts
3. Osteoconduction
4. Mechanical stimulation

Gene therapy augments the first three factors and hence can be propitiously used for Bone repair. Apart from the aforementioned conditions, Temporomandibular joint diseases, craniofacial anomalies, and traumatic amputations can also be treated by introduction of appropriate genes for repair. The main advantage of regional gene therapy for treatment of bony defects lies in the fact that it does not require long-term release of the therapeutic agents. The expression should last till the completion of healing. Bone Morphogenetic Proteins are responsible for skeletal growth and development in all stages of human life. Of particular interest, are the BMPs 2,7 and 7 which are known to induce bone formation in vitro as well as at heterotopic sites. As noted in several clinical trials and animal studies, BMP 2 and 7 are most efficient and effective with respect to their osteoinductive property. Even though individual genes have the ability to induce bone formation, they normally work together. Therefore, transfer of multiple genes can enhance bone repair [1, 9] There are two approaches for regional gene transfer: In vivo and Ex vivo therapy.

In vivo transfer

Based on various studies, BMP-2 and 7gene can be directly delivered via Adenovirus vector for repair of osseous defects. In few animal studies, it was observed that Bone formation was lesser in Immunocompromised compared to Immunocompetent animals. However, due to the use of viral vectors for injection of reparative genes, there is often a low-level immunogenic reaction seen following therapy. This can be prevented or immunized by inducing transient immunosuppression in the subject. [9]

Ex vivo transfer

The cells that are commonly used as carriers for therapeutic gene include mesenchymal stem cells, adipose-derived stem cells, muscle-derived stem cells, blood and skin fibroblasts and buffy coat cells from bone marrow. It has various advantages over using in vivo strategy.

1. Safer; does not involve viral vectors
2. Specific cells can be selected as cellular delivery vehicle depending on the clinical condition
3. Highly efficient cell transduction
4. Bone marrow and stem cells have both autocrine and paracrine functions and hence they can enhance bone repair.

In addition to BMP, other proteins like non-collagenous bone sialoprotein, which is coded by CBFA1 gene also helps in bone regeneration. [3] Similarly, platelet-derived growth factor (PDGF) plays a very important role in wound healing and fracture repair owing to its biological effects on cells. It is a potent activator of cells of mesenchymal origin and causes cell migration, proliferation, synthesis of extracellular matrix and is also anti-apoptotic in nature. It transduces its signal through the macrophages present in the wound. It induces a positive autocrine feedback loop and also triggers an increase in endogenous PDGF and other growth factors which accelerates the wound healing process. [10]

3.3 Therapy for Cancer

Oral cancer is the sixth most common cancer affecting millions of people across the globe. [11] Even though the oral cavity is a readily accessible site, malignant changes are often missed out on visual examination and diagnosis gets delayed to the advanced stage of cancer. There are numerous treatment modalities which have been in use for treatment of this deadly disease. It has been observed that the response rate is higher when patients receive a combination of Gene therapy with the conventional treatment. [3] Local gene therapy gives successful results, owing to the anatomical location of Head and neck squamous cell carcinoma. Injecting into the localized tumor mass precludes the unwanted side effects on the body and also prevents degradation of the gene before it reaches the target cells. Carcinogenesis is triggered either by an abnormality in expression of tumor suppressor genes such as p53, p21, p16, Rb genes or by overexpression of oncogenes like Ras, Myc, Bcl-2 etc.

The various approaches for gene therapy include Gene replacement therapy: It involves correcting the underlying genetic defect to control the multiplication of cancerous cells. The overexpression of oncogenes is controlled by inclusion of DNA which inhibits transcription and translation, thereby halting cell multiplication. Inactivation of p53 gene (tumor suppressor gene) results in a loss of normal apoptotic activity and uncontrolled multiplication of abnormal cells. With the help of gene therapy, the correct copy of this gene is implanted into the tumor cells which not only results in apoptosis but also makes the tumor more receptive to Radiation therapy.

Suicide gene therapy: Gene coding for a pro-drug is introduced into the cancerous cells. The intracellular enzymes act on this pro-drug, converting it into its active form which is toxic to the cells and causes cell death.Oncolytic viruses: These are DNA or RNA viruses which only replicate in cells with a defective p53 pathway, i.e. tumor cells. Adenovirus ONYX-015 is a replication-defective cytomegalovirus that lacks E1B protein, which prevents its replication in cells having a normal p53 pathway. It causes selective tumor cell lysis and causes tumor regression.Gene excision therapy: Early growth factor response factor 1(EGR 1) causes cegrowth and progression of cell cycle. It also results in expression of pro-neoplastic molecules. This protein is inhibited from phosphorylation by introducing Okadaic acid, a toxic polyether molecule, thereby inhibiting cancer cell growth.Immunotherapy: Patients having cancer often present with deficits in their immune response, associated with Natural killer cells, T lymphocytes, dendritic cells. This can either be the result of localized tumor evasion or a generalized immune response deficit. This gene therapy strategy aims to improve the immune response either by administering antigens or adjuvants into the body or by improving the immunogenicity of tumor cells to increase their detection and destruction. The former can be achieved by introduction of one of the following- replication defective cancer cells or purified tumor antigens with or without cytokines or lab-grown antigen presenting cells with or without cytokines as adjuvants. Down-regulation of oncogenic expression: Short chains of anti- sense RNA complementary to the oncogenic DNA nucleotides is implanted. The DNA-RNA complex is identified as a foreign message and is enzymatically cleaved, which reduces the expression of the gene.

3.4 Orthodontic tooth movement

Bone remodeling forms the foundation of orthodontic tooth movement. It involves osteoblasts, which originate from stromal cells and osteoclasts which are derived from hematopoietic cells. Maturation and activation of osteoclasts are partly dependent on its interaction with the cells of osteoblastic lineage. The mediator of this interaction in receptor activator of NF-kappa B ligand (RANKL) molecule. Osteoclastic precursors express RANK receptor on its surface for binding of RANKL molecule, which then converts it into multinucleated giant cells. Osteoprotegerin OPG), which is produced by osteoblasts, competes with the osteoclastic precursors for binding with RANKL, thereby slowing down the process of bone resorption. [1, 3] Local RANKL gene injection in the periodontal ligament increases the local concentration of RANKL molecule, which increases the rate of osteoclastogenesis. This will increase the rate of bone resorption, and thereby reduce the time required for desired tooth movement. [12] Linares et al, [13] experimentally tested the efficiency of selective gene therapy with RANKL as an alternative to corticotomy surgery and concluded that Gene therapy is more effective in terms of accelerating tooth movement compared to the latter. OPG gene can be used to slow down the process of osteoclastogenesis and can inhibit tooth movement by about 50% after 21 days of force application. [3] This can bring about a revolutionary change in the way orthodontics is practiced in the current times.

3.5 Chronic pain management

A major part of dental practice revolves around alleviation and management of pain. Chronic pain is a significant public health concern all around the world, due to its complexity and diversity of conditions that can be responsible for it. Gene therapy may be particularly useful in management of chronic intractable pain. With the help of gene therapy, tissue-specific biological pathways associated with pain can be targeted. Also, long-term sustained analgesia can be achieved, thereby eliminating the need for repeated dosing. Some viral vectors are very commonly used in pain management especially when a specific CNS or PNS dysfunction is targeted. Herpes simplex virus 1 has a natural mechanism of targeting sensory neurons and can be used to achieve gene expression in specific dorsal root ganglia and dermatome distribution. [14] It affects the production of inhibitory neurotransmitters (GABA) or anti-inflammatory peptides to reduce pain. Another approach is intrathecal injection of genes coding for interleukin-10 which has an anti-inflammatory action, using viral vectors or lipid spheres. It transduces the meningeal cells, neurons and glial cells in spinal parenchyma and helps in alleviating pain sensation. [1]

3.6 Tooth regeneration and repair

The best replacement for a lost tooth is tooth itself i.e. a biological implant. Gene therapy can play a very important role in restoration of lost tooth structure due to caries, periodontal disease, and trauma. It makes use of two approaches:

1. In vivo gene therapy: The healing potential of the dentin-pulp complex is enhanced by introduction of genes which induce dentin formation when applied on an exposed pulp. Supernumerary teeth are said to develop from 'third set of dentition', which can be stimulated to naturally form teeth. This can be done by activating genes which code for signaling molecules and proteins responsible for tooth formation.
2. Ex vivo gene therapy: Sources of multipotent dental stem cells are dental pulp, apical papilla, periodontal ligament, deciduous teeth and dental follicle. These stem cells can be procured, cultured in the laboratory, modified to get the desired results and re-implanted back into the recipient.
3. There are 20 basic proteins that are necessary for tooth formation and once all the genes coding for these proteins are tracked successfully, tissue engineering for oral and dental tissues can have promising outcomes in the future.

3.7 DNA Vaccination

Traditional vaccination methods have been used for prevention of dental caries and periodontal disease with limited success. Gene therapy is a novel approach to administer a vaccine, in which, DNA plasmid coding for specific antigens is introduced using eukaryotic vectors. Mucosal administration of vaccine using liposomes has shown enhanced mucosal immunity and promising resultsColonization by S.mutans and other organisms is one of the key factors responsible for dental caries. Anti-caries DNA vaccination evokes a strong humoral and cellular response against S. mutans associated antigens and also has a low immunogenicity.[15] Plasmid coding for pac gene(major cell surface adhesion), introduced into targeted salivary glands has shown significant immunization in rat models. [1] Porphyromonasgingivalis is the principle microorganism responsible for periodontitis. It has been seen that specific genes control the pathogenicity of this organism. rgpA gene codes for pathophysiologically significant proteins which results in periodontal tissue destruction. Immunization using rgpA gene can result in a considerable reduction in periodontal tissue break down. [1] This concept can also be extended to develop mutant strains which are less infective and can thereby control periodontal disease progression. [5]

IV. Conclusion

Even though gene therapy is still at its stage of inception, it can revolutionize the way we practice dentistry today. It can act as a significant tool in improving the quality of care delivered to the patient by providing customized tailor-made treatment. The oral cavity acts as a window to the rest of the body, and since it is a readily accessible site, gene therapy can be accomplished in a minimally invasive manner. It is an active area of research for a wide range of dental and biomedical applications like therapy for chronic intractable pain, Oropharyngeal cancer, Salivary gland disorders, DNA vaccination, bone repair and tooth regeneration. There are a few challenges with respect to ethical and safety issues, cost-effectiveness, vector associated toxicity and pathogenicity which can be overcome in the near future with further research and development in the field of gene therapy and tissue engineering.Refrecingand thanbewvarifacation

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