ISSN: 2581 – 3633
PubMed - National Library of Medicine - ID: 101745081

International Journal of Medical Science and Advanced Clinical Research (IJMACR)

Available Online at: www.ijmacr.com

Volume – 5, Issue – 2, March - April - 2022, Page No. : 179 - 188

Gene therapy in oral diseases

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How to citation this article: Deepak Narang, "Gene therapy in oral diseases", IJMACR- March - April - 2022, Vol – 5, Issue - 2, P. No. 179 – 188.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Gene therapy holds a promising future for bridging the gap between the disciplines of medicine and clinical dentistry. The dynamic treatment approaches of gene therapy have been advancing by leaps and bounds. They are transforming the conventional approaches into more precise and preventive ones that may limit the need of using drugs and surgery. The oral cavity is one of the most accessible areas for the clinical applications of gene therapy for various oral tissues.

The idea of genetic engineering has become more exciting due to its advantages over other treatment modalities. For instance, the body is neither subjected to an invasive surgery nor deep wounds, nor is it susceptible to systemic effects of drugs.

Gene therapy involves transfer of new genetic material or manipulation of the existing material for the purpose of treating human disease. It involves transfer of genetic material – ex vivo and in vivo and may conceivably revolutionize clinical practice in the coming years.

Although progress has chiefly been in the medical domain, research has moved to various dental conditions as well. This review is presented to highlight various applications of gene therapy in dentistry in the areas such as salivary gland disorders, chronic pain, DNA vaccines, bone repair, implantology, head and neck cancer, orthodontic therapy, periodontal repair and tooth regrowth.

The aim of this article is to review the gene therapy applications in the field of dentistry. In addition, therapeutic benefits in terms of treatment of oral diseases, minimal invasion and maximum outcomes have been discussed.

Keywords: Gene Therapy, Gene, Mutations, Oral Diseases

Introduction

Gene therapy deals with replacing the defective genes with their correct analogues to produce functional proteins. Evidence suggests that gene therapy can be used to prevent, alleviate or cure underlying disorders including cancers, infectious diseases, and genetic and autoimmune disorders[1].

A gene therapy medicinal product is defined as any biological product consisting of an active component of recombinant nucleic acid used to regulate, repair, replace, enhance or delete a genetic component.

However, vaccines against infectious diseases are not classified as gene therapy medicinal products[2].

In terms of functioning, every gene stores information for synthesis of a specific protein that in turn controls various bodily functions and mechanisms. Any defect in the gene, therefore, results in an inevitable aberration in the physiology and results in development of diseases[1].

Gene therapy is being considered as an innovative treatment modality for the correction of faulty genes by genetic modification of the human cells and subsequent formation of functional proteins.

Gene therapy is a two-step process; in the first step, human genetic coding for the therapeutic protein is first cleaved and inserted into the genome of a carrier or vector that is usually an attenuated virus. In the second step, the entry of modified vector to the target human cells results in releasing the DNA sequence that becomes integrated within the chromosome.

As the gene is “switched on” in its correct location, the cells with the new genetic design start forming the required therapeutic proteins[3,4]. In general, the gene therapy can be classified into two distinct stages somatic and germ line gene therapy.

The somatic gene therapy involves changes in the target cells, however, that are not transferred to the next generation. In contrast, with germ line gene therapy, the modified genes are transferred to the next generation.

There are certain ethical issues regarding germ line gene therapy; hence, somatic cell gene therapy is currently allowed. Although germ line gene therapy offers possible treatment for hereditary disorders in the future, due to ethical issues, it has been limited to animal models. Besides ethical concerns, the risk of unexpected damage to the developing fetus[5] is also a major concern obstructing the use of germ line gene therapy for human clinical trials.

The characteristics of an ideal vector include high specificity, low virulence and its ability to completely unload the normal human gene into the host cells. Viral vectors are more efficient in transferring the gene but carry a risk of causing illness.

Most popular viral vectors include adenoviruses and retroviruses. Non-viral vectors are cheaper, safer and can deliver large amounts of DNA into the host cell. However, efforts are being made to improve their transfection rate[6,7,8].

History

The concept of genetic therapy has been known for many decades. The idea of transforming principles was introduced by Griffith in 1928 during his experimentation on pneumococcal bacteria. The role of deoxyribonucleic acid (DNA) to purify the transformed genetic products was described in 1944 followed by the idea of transduction through transformation of genetics in bacteria in 1952.

During the 1950s and 1960s, researchers focused on understanding the double helix structure and role of DNA for genetic therapy. Further research led to conduction of the first ever human gene therapy trial in 1973.

In 1990, the Food and Drug Administration (FDA) approved gene therapy for therapeutic applications in the United States. In the last decade (2001–2010), a number of genetic products such as Gendicine™ (Si Biono Gene Tech, Shenzhen, China) and Cerepro ™ (Ark Therapeutics Ltd, London, UK) have been developed and approved for therapeutic applications and have become commercially available.

Further research is in progress to develop more products for therapeutic applications focusing particularly on malignant lesions and autoimmune diseases[9].

Gene therapy in dentistry

Since the introduction of gene therapy for dental applications, remarkable progress has been made in the field of genetic therapy for a range of applications in dentistry (Figure 1).

In order to improve the quality of life, gene therapy has promising outcomes for potential treatment for multiple disorders and has been discussed in this review[10].

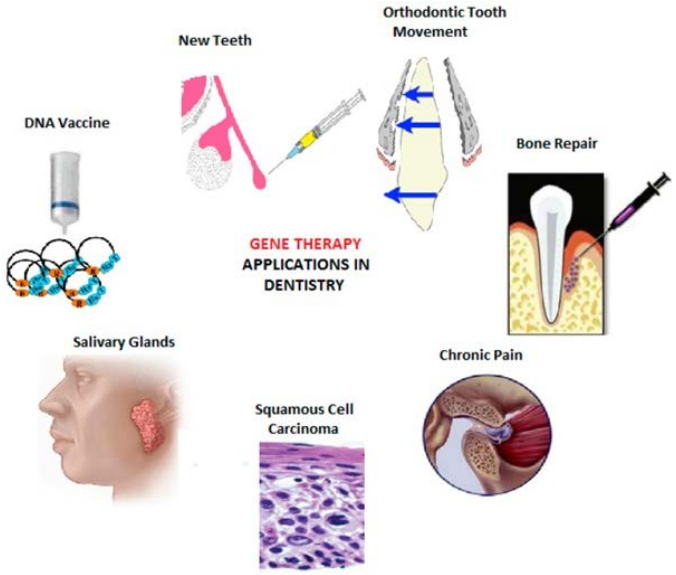

Fig. 1: Gene therapy in dentistry

Gene therapy in orofacial pain

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, 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

Orofacial pain refers to the pain associated with hard and soft tissues of the face, head and neck region. The pain impulses are conducted through the 5th cranial (trigeminal) nerve to the central nervous system.

Due to the diffuse and referral nature, the diagnosis and management of orofacial pain remains a major challenge. Patients commonly seek treatment for common causes of orofacial pain such as temporomandibular disorders (TMD)[11].

The orofacial pain may originate from dental hard tissues (pulpitis, hypersensitivity), oral soft tissues (Temporomandibular joint, glands, orthodontic), neurological tissues (neuralgia) and vascular or psychogenic tissues.

Conventionally, the pain management involves using analgesics and sedatives. Gene therapy is being investigated for improving management of chronic pain by sparing the use of drugs with the associated risk of systemic toxicity, opioid addiction and other side effects [12,13].

The continuous production and secretion of anti-nociceptive proteins in or near spinal dorsal horns may be achieved in two ways. Firstly, the modified adenovirus, adeno associated virus (AAV) or lipid encapsulated plasmids coding for Interleukin-10, a therapeutic protein, may be injected into the sub arachnoid space to transduce the pia mater cells[14].

Secondly, a modified herpes virus may be introduced into the nerves of Dorsal Root Ganglia (DRG) via an intradermal injection to the skin. The rationale for using the herpes virus is that it infects nerves, and it therefore has the ability to travel to the DRG via nerve endings in the skin. In the DRG, it codes for an inhibitory

neurotransmitter, an anti-inflammatory peptide or decreases the synthesis of an endogenous nociceptive molecule that results in alleviation of pain [15].

At present, the use of gene therapy for alleviation of pain is mainly limited to animal models. Recently, a reduction in trigeminal pain has been reported encoding the human PR proenkephalin gene through a herpes simplex vector in a mouse model[16].

Gene therapy may offer a dose of hope to the pain therapist in treatment of pain syndromes such as trigeminal neuralgia and temporomandibular joint disorders with improved vector systems in the future [17,18].

Gene therapy in salivary gland pathology

Tumours and their surgical resection, autoimmune disorders such as Sjogren's syndrome, post radiation 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

Salivary glands secrete saliva that has a physiological role for lubrication, mastication and digestion of food. Saliva is rich in antimicrobial peptides that are a vital component of local immunity. The lack of salivary gland functions results in dryness of the mouth (xerostomia).[19]

Common etiological factors of xerostomia included salivary gland impairment, radiotherapy of the head and neck, autoimmune disorders (such as Sjogren′s syndrome) and certain medications. Salivary glands exhibit several important features such as: self-containment due to a surrounding capsule that is likely to minimize the undesirable access of administered vectors and transgenes to other tissues, and highly efficient protein production.

Their ability to secrete proteins into the bloodstream makes them potentially useful target sites for gene transfer in a minimally invasive manner with the help of intraductal cannulation[20].

With the origin of Aquaporin 1 gene, it is anticipated that patients suffering from hypofunctional salivation due to ionized radiation will soon be cured with the help of gene therapy. These ionized radiations cause severe damage to the fluid secretory portion (acinar cells) of the salivary gland that lies in the field of radiation[21].

Aquaporin 1 (AQ1) is a water channel protein that counterbalances this detriment by a constitutively activated water channel. In the irradiated submandibular glands of a rat and the in parotid glands of an adult rhesus monkey, it showed positive results [22].

A recent clinical trial has advocated the safety of using AQP1 gene therapy for the management of xerostomia patients Patients undergoing radiotherapy usually develop xerostomia due to irreversible acinar damages. The successful human clinical trials illuminated hopes for clinicians to overcome radiotherapy related salivary hypo functioning in the near future. However, the use of AQP1 gene therapy for the management of radiotherapy related xerostomia may involve a few challenges such as

prevention of host immune reaction and repeated administrations throughout the course of treatment. Further clinical trials are required to be conducted to decide if it is useful to pursue the AQP1 gene transfer strategy clinically[23].

Gene therapy has been augmented in the treatment of salivary glands along with other systemic conditions. Secretory gene proteins are injected into salivary glands, which are secreted in an exocrine manner. This gene transfer mechanism results in treating disorders of the mouth and upper gastrointestinal tract. Researchers have injected a naturally occurring salivary anti-candidal polypeptide human histatin 3 (H3)) into the azole resistant candidiatic submandibular gland of rats. Copious amounts of H3 polypeptide were secreted in saliva that killed both azole-sensitive and azole-resistant Candida species with approximately equal efficiency [24].

Gene therapy in tooth repair and regeneration

Tissue engineering has been developed for the regeneration and repair of tissues Research has reported promising outcomes for the tissue engineering of various oral and dental tissues [25].

Gene therapy presents an attractive concept of restoring the oral tissues lost due to caries, periodontal diseases and trauma. This could widen the scope for development of new teeth—the biological implants for missing teeth. This makes use of the two basic approaches including in vivo and ex vivo gene therapy.

In vivo gene therapy, the healing potential of tissues such as dentine pulp complex, is enhanced by genes stimulating dentine formation after being applied directly on the exposed dental pulp[26]. Many people have supernumerary teeth that arise from the third set of dentition. This third dentition can also be induced to form teeth in a natural way by turning on or activating genes which code for proteins and signaling molecules making up the basic structure of teeth[27].

Gene therapy in orthodontic tooth movement

Orthodontic tooth movement is possible due to remodeling of periodontal ligament and alveolar bone that is controlled by osteoclasts and osteoblasts. Precursors of osteoclasts are hemopoietic cells, whereas osteoblasts originate from stromal cells.

Maturation and activation of osteoclasts require interaction with cells from the osteoblastic lineage. The molecules mediating such interactions are the receptor activator of the nuclear factor kappa B (RANK) or receptor activator of nuclear factor kappa-B ligand (RANKL) Osteoclastic precursors express on their surface RANK, the receptor for RANKL that binds and converts them into multinucleated giant cells.

Osteoprotegerin (OPG), a soluble receptor produced by osteoblasts, is in competition with the RANK receptor binding to RANKL.

Upon binding with RANKL, it inhibits osteoclastogenesis, thus jamming the process of bone resorption. Two significant studies were made by using gene therapy with OPG and RANKL to speed up and impede orthodontic tooth movement in a rat model[12,13].

Gene therapy in DNA vaccination

An effective caries vaccine or periodontal vaccine has been an elusive dream, with many researchers only achieving mixed success. Instead of delivering an attenuated microbe or isolated protein to the immune system, as was attempted earlier, DNA transfer of specific genes of selected microbes to salivary tissue has been experimentally attempted.

In the study, Porphyromonas gingival is fimbriae antigen was expressed by salivary cells after gene transfer resulting in the formation of antigens of s-IgA and IgG

classes against the microbe, which would target the periodontal pathogen.

Additionally, the antigen would compete with the live organisms for attachment in dental plaque. Similar DNA vaccine attempts have been made for Mutans Streptococci as well[28]

Gene therapy in oral cancer

Gene therapy in cancer treatment broadly aims at expressing a gene product that will result in cancer cell death. This may be accomplished by various strategies:

A. Addition of a tumour suppressor gene (gene addition therapy) e.g. P53 in cancer cells,

B. Deletion of a defective tumour gene (gene excision therapy),

C. Down regulation of expression of genes that control tumour growth,

D. Enhancement of immune surveillance,

E. Activation of pro drugs that have a chemotherapeutic effect and cause toxicity only to the tumour cells ('suicide gene' therapy),

F. Antiangiogenic therapy to inhibit tumour angiogenesis,

G. "cancer vaccination" with genes for tumour antigens[29].

Gene addition therapy

The p53-expression alteration has been widely implicated in a variety of cancers as an early event in carcinogenesis, and p53 tumour suppressor gene addition has been attempted using a variety of viral vectors in various animal and clinical trials to trigger a pro-apoptotic anti-tumour effect.[30]

Retroviral, adeno-associated viral, herpes viral and adenoviral vectors have been tried, and of these adenoviral vectors have proven more successful than others, as they are capable of gene addition in both dividing as well as resting cells, without any permanent additions to the host genome. Even more successful has been the use of oncolytic viruses, which are designed to replicate only in cancerous cells lacking p53, to produce tumour regression in refractory cancers or to reduce or produce precancerous dysplastic lesion regression[31]

Gene-excision therapy

Gene-excision therapy is another strategy to inhibit the growth of cancer cells. Here, early growth factor response factor 1, which controls cell growth and cell cycle progression including the expression of pro-neoplastic molecules such as TGF-β1, PDGF-α, is inhibited from phosphorylation by the action of Okadaic acid, a toxic polyether molecule[32]

Suicide gene therapy

The addition of suicide genes by viral vectors is another strategy for treating carcinomas where genes coding for a pro-drug are transfected to cells, via herpes virus or adenovirus. When these enzymes are synthesized intracellularly, they metabolize drugs which trigger cell death. The Herpes Simplex Virus-Thymidine Kinase (HSV-TK) triggers metabolism of ganciclovir to ultimately terminate DNA-synthesis

Gene therapy in periodontal management

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) have been made commercially available and biomolecules expressed during dentinogenesis such as Odontogenic Ameloblast Associated Protein (ODAM), Amelogenin, OP-1, TGF-β, BMP-4 and BMP-2 have been shown to work experimentally by direct instillation. However, a matter of concern is that several of these

biomolecules favour osseous healing which may predispose to ankylotic healing of periodontal tissues[33,34,35].

Advantages of gene therapy

- A functional gene has the ability to replace a defective gene.
- Gene therapy aids in the prevention against the potentially toxic effects in the body, which can be caused by other therapies.
- It decreases the cost of various therapies and improves the patient's life style for a longer period

Disadvantages of gene therapy

- Even after the therapeutic DNA is integrated into the genome, some cells prevent the gene therapy for long-term effects, for which patients may undergo multiple rounds of the gene therapy.
- ii. There is a possibility that the host's immune system and its response may reduce the effectiveness of the gene therapy.
- iii. The viral vectors can present a variety of potential problems to the patient, such as toxicity and immune and inflammatory responses.
- iv. Single gene disorders are the best candidates for gene therapy. But, some of the most commonly occurring diseases, such as heart disease, high blood pressure, Alzheimer's disease, arthritis, and diabetes are multi-gene or multi-factorial disorders which are difficult to treat effectively with the use of gene therapy.
- v. If the DNA is introduced into a wrong place in the genome, for example, into a tumour suppressor gene, it can induce a tumour.

Future perspective

While single nucelotide polymorphisms and single gene disorders are amenable to detection, analysis and genetic manipulation, the vast majority of traits and disorders are polygenic. In addition to involving numerous genes acting in concert, these genes are also situated at different loci. Further, susceptibility to such traits amongst individuals is dissimilar due to environmental factors exerting varying degrees of influence.

Also, genetic etiology for particular trait may be associated not only with disease causing genes but even with unrelated genes. Unravelling these complex interactions between genes is the biggest hurdle to making personalised medicine realisable[36]

Conclusion

Gene therapy has the potential to disrupt existing therapies or create therapies where none previously exist and undoubtedly, will revolutionize dentistry in the years to come.

This review has presented some of the current approaches in gene therapy in dentistry. Clinical success is already apparent in several human trials and each success inches towards a future of predictive, preventive, personalised medicine.

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