INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND PHARMACEUTICAL RESEARCH

IJCMPR

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RESEARCH ARTICLE

FUTURE PROSPECT OF GENE THERAPY FOR POTENTIALLY MALIGNANT AND MALIGNANT DISORDERS OF ORAL CAVITY"---ITS APPLICATION IN DENTISTRY

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ARTICLE INFO

Article History:

Received 5th November, 2016
Received in revised form 17th December, 2016
Accepted 26th January, 2017
Published online 28th February, 2017

Key words:

Gene therapy, viral vectors, non viral vectors, dentistry, oral cavity.

ABSTRACT

Background- Gene therapy comprises of introducing specific genetic material into target cells without producing toxic effects on surrounding tissue. Gene therapy is one of the recent advances in this field to deal with this obstacle with promising prospects. As an oral physician it is our responsibility to treat common oral premalignant lesions and conditions like leukoplakia and oral submucous fibrosis which have a definite malignant potential if timely and early diagnosis and treatment is not provided. Treatment options for recurrent or refractory oral cancers are limited.
Material and methods- A Medline search was done including the international literature published between 1989 and 2013. It was restricted to English language articles and published work of past researchers including in vitro and in vivo studies. Google search on gene therapy was also done.
Results- Despite advances in surgery, radiotherapy, and chemotherapy, the survival of patients with oral squamous cell carcinoma has not significantly improved over the past several decades. A potential approach to the treatment of oral premalignant and malignant disorders is gene therapy.
Conclusion-This article highlights the principle, transfer techniques, therapeutic strategies, clinical applications and limitations of gene therapy in the treatment of disorders pertaining to head and neck region.

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INTRODUCTION

A gene is a molecular unit of heredity of a living organism. It is a name given to some stretches of DNA and RNA that code for a polypeptide or a RNA chain having a function in the organism. Genes hold the information to build and maintain an organism's cell and pass genetic traits to the offspring [1].

The goal of gene therapy (GT) is to introduce therapeutic genetic material into the target cell to exert the intended therapeutic effect. It has shown promising results for the treatment of many disorders afflicting the human body [2]. It has been introduced as a potential approach for the treatment of oral premalignant and malignant disorders.

According to Cusack and Tanabe (1998), (GT) includes the transfer of the new genetic material as well as the manipulation of the existing genetic material for the purpose of treating human diseases [3].

The first gene therapy trial on humans was done on patients with severe combined immunodeficiency syndrome (SCID) in the year 1990 [4]. First successful gene therapy trial in patient with functional immune system was performed in the year 2000. [4].

Methods of Literature Search

The literature was searched using Pubmed and electronic data bases from 1999 to 2011. Key words such as gene therapy, dentistry, were used. The search was restricted to English language articles, published from 1999 to 2013, and various in vivo and in vitro studies were included. The purpose of this review was to highlight the application of gene Therapy use of various viral and non viral vectors in various fields of dentistry, with emphasis on its possible future prospects.

Gene delivery systems[5, 6, 7]

There are two methods for inserting genetic material into human chromosomes:

1. Ex vivo technique: involves surgically removing cells from the patients, injecting or splicing the new DNA into the cells and letting them divide in cultures and placing them into the affected area.
2. In vivo technique: involves direct injection of therapeutic DNA into the body cells.

A Vector is a carrier molecule used to deliver the therapeutic gene to the target cells. The most common vectors that have been genetically altered to carry normal human DNA are viruses, which encapsulate and deliver their genes to human cells in a pathogenic manner. The vectors then unload their

genetic material containing the therapeutic human gene into the target cells. The ideal vector transfers a precise amount of genetic material into each target cell, thereby allowing for expression of the gene product without causing toxicity. There are two methods:

Viral vector

Viruses replicate by binding to the host cell, viral genome is integrated into the host cell, which multiples and the viral genome replicates and multiple copies of virus infected cells are produced. Viruses can thus be manipulated to carry the functional gene into the diseased cell and the abnormal gene is replaced by the functional gene [7]. Examples: retroviruses, adenoviruses, adeno-associated virus and herpes simplex viruses.

These viruses can infect only actively dividing cells, leaving quiescent cells unaffected. This has been overcome by the use of lentiviral vectors, which activate the immune system in pre-clinical animal models of oral cancer.

Non Viral Vectors

Physical vectors include electrophoration, microinjection, use of Ballistic particles.

Chemical vectors include calcium vectors, lipids, protein complexes.

Electrophoration: Electrical current creates transient holes in the cell membrane through which DNA can be transferred.
Microinjection: DNA is introduced in a single cell.

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.

Calcium Vector: The ultra-low size, highly monodispersed DNA doped calcium phosphate nano particles protected from the external DNAse environment are used to transfer the encapsulated DNA under in vitro and in vivo condition.

Lipid Vectors: produced by a combination of plasmid DNA and a solution resulting in the formation of liposome. This fuses with the cell membrane of a variety of cell types, introducing plasmid DNA into the cytoplasm.

Genetic principle are been applied along with tissue engineering for rehabilitation of tissue.

Tissue engineering can be categorized into three major classes [4,7].

1. Conductive: Biomaterials facilitate the growth of existing tissue in a passive manner. E.g. the use of barrier membranes in guided tissue regeneration. Nyman *et al* was the first to use osteoconductive mechanism in providing amines for selective wound healing by supporting the in-growth of periodontal supporting cells.
2. Inductive: Inductive cells in close proximity to the defect site with specific biological signal. Urist first reported the formation of new bone at ectopic sites after implantation of powdered bone.

Table No 1 Advantages & Disadvantages of viral vectors

Vectors	Advantages	Disadvantages
Viral Retroviruses	Well known, easily managed Includes upto 9 kb of exogenous material Randomly integrate into host genome long term genetic alteration Integration in genome ensure permanent expression	Infects only cells in division Low transduction efficacy Possible insertion mutagenesis Low titres
Adenovirus	Infect both dividing and quiescent cells Includes upto 7.5 kb of exogenous genetic material High transduction efficiency Does not integrate in genome which avoids insertion mutation	Transient expression Anti-adenoviral immunity can lessen effect Lowers expression level possible immune and inflammatory reaction
Adeno associated viruses	High transduction efficiency Low immunogenicity	Difficult to manufacture Low titre

Table No 2 Application of Gene Therapy in Dentistry

Periodontal vaccination	TGF-β, BMP-2, 6, 7, 12, bFGF, VEGF, PDGF
Bone	BMP-2, BMP-7
Skin and oral mucosa	Human factor IX,Human Apolipoprotien E , Growth hormone ,IL- 10,gene activated matrix (GAM)
Salivary gland	Histanin 3 or H3, aquaporin 1 aquaporin 5, transcription factor (E2F-1), protease inhibitor (α-1 antitrypsin and kallistanin) "Repoter Proteins" β galactosidase
Dental pain	Viral mediated transfer of genes encoding opiate peptides
DNA vaccination	Dental caries and Periodontal disease.
Tooth development	Homeobox gene, PAX-9 Gene, MSX-1 gene, DLX gene, LHX gene, BARX gene, RUNX gene
Syndrome associated with orofacial defects	Mutaed EDA gene
Ectodermal dysplasia	Mutated GJB-6 gene
Witkrop tooth and nail syndrome	Nonsense mutation within MSXI homeobox gene
Reiger syndrome	Malformation in PITX2 a gene mapped to 4q25-q26, PITX-2
Structural tooth defects	AMELX & ENAM
Amelogenesis imperfecta Dentinogenesis imperfecta	COLIA I and COLIA-2 gene
Vander Woude Syndrome	Mutation in IRF-6 gene
Crouzon Syndrome	Mutation in FGFR-2 gene
Apert Syndrome	Mutation in FGFR-2 gene
Treacher Collins Syndrome	Haploinsufficiency of TCOF-I gene
Down Syndrome	Trisomy 21
Oral premalignant condition- Leukoplakia, Oral submucous fibrosis	Advexin (INGN 201,Ad5CMV-p-53)
Malignant condition	HLA-B7,ONYX-015, HSV-Tk, RGD peptides,OAS403
Squamous cell carcinoma	Adv-F/RGD

3. Cell transplantation: It reflects the multidisciplinary nature of tissue engineering, as it requires the clinician or surgeon, the bioengineer and the cell biologist.

Application of gene therapy in dentistry

1. Periodontal vaccination
2. Bone
3. Cartilage
4. Skin and oral mucosa
5. Dentin and dental pulp
6. Salivary gland
7. Genetic approach to film antibiotic resistance
8. In vivo gene transfer by electroporation for alveolar remodelling
9. Antimicrobial gene therapy.
10. Designer drug therapy in treating periodontal disease.
11. h) Oral premalignant and malignant lesions of oral cavity.

The Gene involved in Tooth Development[8]: Tooth development is an example for the reciprocal interaction between ectoderm and underlying mesenchyme, resulting in sequential cell activities like proliferation, condensation, adhesion, migration, differentiation, and secretion and formation of a functional tooth organ. Recent advances in molecular aspects of odontogenesis indicate that the development of teeth is under strict genetic control. More than 300 genes are involved in determination of the position, number, and shape of different types of teeth.

Homeobox Genes

Most commonly studied genes in tooth development are homeobox genes. It is a DNA sequence of about 180 base pairs long, found within genes involved in the regulation of development of animals, fungi, and plants. Humans contain these genes in 4 clusters, called HOXA, HOXB, HOXC, or HOXD on chromosomes 2, 7, 12 and 17. PAX, MSX, DLX, LHX, BARX, and RUNX-2 are the important members of the homeobox genes involved in tooth development.

PAX-9 gene

This genes belongs to a transcription factor family with 9 members characterized by a DNA-binding domain called paired domain. PAX-9 is widely expressed in the neural crest derived mesenchyme involved in craniofacial and tooth development. PAX-9 is required for the mesenchymal expression of Bmp-4, MSX-1, Lef-1, suggesting that its function is essential to establish the inductive capacity of this tissue.

MSX-1 gene

It is a member of MSX homeobox gene family related to the drosophila gene muscle segment homeobox (msh), expressed in undifferentiated multipotential cells that are proliferating. MSX-1 regulates epithelial-mesenchymal signaling and provide positional information in cranio-facial development.

DLX gene

DLX (Distal less) family of homeobox genes consists of 6 members (DLX 1-6) and is expressed in the epithelium and mesenchyme of the branchial arches, tooth bud mesenchyme, dental lamina, cranial neural crest, dorsal neural tube, and frontonasal process. Any mutation in these genes results in abnormalities affecting first four branchial arch derivatives including mandible and calvaria and in failure of development of upper molars.

LHX gene

Lim homeodomain transcription factors (LHX-1 and LMX1-b) are expressed in neural crest derived ectomesenchyme of first branchial arch. Abnormal development of first arch derivatives including tooth agenesis and cleft palate occurs due to improper expression of this gene .

BARX gene

BARX gene is expressed in Telencephalon, diencephalon, mesencephalon, hindbrain, spinalcord, cranial and dorsal root ganglia, craniofacial structures, and palate. BARX-1 is expressed in the mesenchyme of the mandibular and maxillary process and in the tooth primordial, while BARX-2 is expressed in the oral epithelium prior to the tooth development.

Improper expression results in failure of nervous system development and cleft palate formation.

Bone repair: Studies by researchers at the University of Michigan School of Dentistry have used ex vivo methods to transfer genes encoding bone morphogenetic proteins (BMPs). BMPs are agents established in induction of both orthotopic and ectopic bone formation[9]. In animal models, the Michigan research group has shown that several different cell types-such as nonosteogenic fibroblasts and myoblasts, as well as osteoblasts, which are able to differentiate into bone-forming cells when placed in an osseous defect in vivo [10].

Salivary glands [9,11]- Salivary glands are capable of producing large amounts of proteins and are sites where gene transfer can be readily accomplished in a minimally invasive manner. Gene therapy helps in treating irreversible salivary gland dysfunction resulting from irradiation or the autoimmune damage occurring with Sjögren's syndrome. A variety of genes can be transferred including genes encoding hormones (growth hormone, insulin), antimicrobial agents (histatin 3, or H3),15membrane proteins (aquaporin-1 and aquaporin- 5), transcription factor (E2F-1), protease inhibitors (α1-antitrypsin and kallistatin), a\protein affecting apoptosis (Fasligand)[10,11]. The efficacy of An adenovirus-encoding human AQP1, termed "AdhAQP1," was administered to hypofunctional rat submandibular glands [12] and then later on rehus monkeys[13] .The result was not as encouraging as in monkey model as in rat model maybe due to the fact that in monkey the glands were undefiled by vectors. Thus additional animal studies are required if it is useful to pursue the AQP1 gene therapy strategy clinically.

Skin and Oral mucosas[10,11]- The engineering and transplantation of oral mucosa and gingiva could be potentially important as a new technique in periodontal graft surgery and the treatment of gingival recession. The main aim is to transfer foreign genes into mucosal and epidermal keratinocytes for normalization of tissue structure and epidermal function using retrovirus.

Keratinocytes are very favorable in these sites, some of its advantage being:

1. The area is easily accessible so monitoring is adequate.
2. Preclinical assessement is accurate.

3. Expression of therapeutic genes can be achieved with the use of topically applied agents.
4. It is reversible because genetically modified tissue can be excised.

Dentin and Dental Pulp [4,9,10,14]- The lost odontoblasts can be induced from pulp tissue using certain BMP's .Tissue engineering of dental pulp itself may also be possible using cultured fibroblasts and synthetic polymer material.

Pain[15,16] Gene transfer may be 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.

DNA vaccinations --- The use of DNA vaccine was demonstrated in an animal study published in 1999 by Kawabata, Japan[17]. They achieved a targeted salivary gland immunization using plasmid DNA encoding the Porphyromonas gingivalis fimbrial gene, which led to consequent production of specific salivary antibodies IgA, IgG and serum IgG antibodies. Secretory IgA secreted in saliva could neutralize P. gingivalis and limit plaque formation.

Autoimmune disease and gene transfer gene transfer has been used with considerable success for treatment of autoimmune diseases. The biological factors that enhance Th2 functions and suppress Th1 cells are likely will be efficacious for therapy. [18] Recombinant adeno-associated virus, or AAV, serotype 2, are the vectors used for autoimmune disease including SS.[18] HIL-10 immunomodulatory therapy has been tried and shown to be useful in preclinical models of other autoimmune diseases, including rheumatoid arthritis.[19]

Premalignant lesions

Oral leukoplakia: is a well-recognized precancerous lesion, associated with abnormal p53 expression leading to high risk of malignant transformation. Yi Li evaluated the feasibility, safety and biological activity of multipoints intraepithelial injections of recombinant human adenovirus-p53 in 22 patient with dysplasic oral leukoplakia and found it to be successful as compared to other traditional therapy.[20]

Recombinant adenovirus-p53 could strongly inhibit cell proliferation, induce apoptosis, and arrest cell cycle in stage G(1) in POE-9n cells by inducing p21(CIP/WAF) and down regulating bcl-2 expression. But various side effects after Ad-p53 injections was noticed which included transient fever , flu-like symptoms, and increase WBC number which was the inflammatory response shown by viral vector. [20]

The effect of this research provided a new way for the research clinical application.

Patients with precancerous lesions were treated with Advexin® mouthwash (Introgen Therapeutics, Inc (INGN), NY), which also administers p53 by means of an adenovirus. Tissue analysis before and after treatment showed a marked decrease in the number and aggressiveness of precancerous cells.[21]

Oral submucous fibrosis (OSMF) A study was conducted to find out the expression of aberrant p53 in OSMF and Oral squamous cell carcinoma (OSCC) to study correlation between p53 expression and degree of dysplasia in OSMF and OSCC by Sultana N *et al* in 2011. The results indicated that p53 over expression may play a role in the development of Oral squamous cell carcinoma and in pathogenesis of OSMF patients.[22]

Current gene therapy in treatment of OSCC includes:
Addiction gene therapy[6]

It regulates tumour growth by introducing tumour suppressor genes that inactivate carcinogenic cells. 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 developmet of tongue cancer and may in future prove useful for treatment of OSCC [23]. NF-kB appears to contribute towards the progression and metastasis of various cancers, including OSCC, therefore its inhibition may be a useful coadjuvant treatment in oral cancer therapy[24]. 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 [25].

Recent mouse studies found that the intratumoural injection of Allovectin-7® (Vical Inc, San Diego, USA), suppressed the growth of head and neck tumours. However, further studies are required since only preclinical trials in mice have been performed to date [26].

Gene therapy using oncolytic viruses[6]

One of the most promising gene therapy approaches is the use of viruses that replicate only tumour cells, designated oncolytic viruses. The release of oncolytic herpes virus in primary tumor after surgical excision has been shown to significantly reduce tumor and regional metastasis [27].Combination of ONYX-O15 and 5-fluoracil increase the efficacy and enchances the activity [28]. Presence of cytokines IL-6, TNF-α, IL-10 and IFN-γ is observed at 24 hrs after ONYX-015 administration, thereby contributing towards the appearance of an immune response to the tumour [29].

Rudin *et al*[21] tested three different dose of advexin in 19 patients with epithelial dysplasia. After a 30-month follow-up, complete remission was recorded for one of the three patients who received the highest dose of the virus (mouthwash) for the first 5 days followed by weekly administration for 5 weeks. Response of p53 function was assessed by determining p53 protein expression, which was significantly suppressed in patients who responded to treatment but not in non-responders.

Suicide gene therapy[6]

"Suicide" gene therapy involves the introduction of a gene into a cell that enables a prodrug to be activated into an active cytotoxic drug[5,6] . When suicide genes are used in retroviral vectors, protection measures must be taken against the appearance of oncogene-activating mutations. The thymidine kinase gene of Herpes Simplex Virus (HSV) transforms ganciclovir into ganciclovir phosphate[30]. Gene transfer of HSVtk gene (Herpes simplex virus thymidine kinase gene) via adenovirus vector in combination with ganciclovir administration may be a good therapeutic option for OSCC[31] Invitro study conducted by yamamoto el [32] suggested that adenovirus –media suicide gene therapy induced reprokable cytotoxic effect in human OSCC can be used as a effective treatment. One of the main drawbacks of suicide gene therapy is the poor distribution of the vector within the tumour due to low transfection efficacy, a high percentage of transfected

cells do not appear in vivo due to their ability to induce tumour death in the neibhboring untransfected cells[5].

Inhibition of tumour angiogenesis

This incorporates the use of microencapsulated cells in cancer therapy [6]. Microcapsules are designed to be permeable to recombinant products and nutrients but not to host immune mediators, due to their large size [6]. Angiostatin receptors present ATP synthase on the surface of human endothelial cells, which enables angiostatin to be localized in the tumour instead of organs near the implantation site of the capsule [6]. However, it is a prolonged therapy that requires repeated doses and is associated with a high degree of toxicity. Vaccines against receptor 2 of the VEGF factor (Vascular Endothelial Growth Factor), result in inhibition of angiogenesis, tumour growth and metastasis, stimulating T lymphocytes. It is useful in the treatment of tongue metastasis of OSCC, with an increased immune response observed at 10 months of the inoculation [32].

Increase in immune response

The main aim of immunotherapy is to increase the patients immune response to tumor. Altered function of immune cells, including NK cells, T lymphocytes and numerous cytokines have been seen in patients with OSCC. Animal studies have shown that IL-2 administration activates T lymphocytes and NK cells and these activate TNFα [33]. Radiosensitivity to γ radiation and chemosensitivity to 5-fluoracil (5-FU) were reported in OSCC after suppression of NF-kB activity, which activates the antiapoptotic proteins TNF, TRAF-1, TRAF-2 and cIAP-1. NF-kB has been shown towards the progression and metastasis of various cancers including OSCC [24].

Other studies have addressed the transduction of IL-2 gene, which appears to have an anti-tumour effect, by using the mutated fibroblast of an adenovirus and an RGD peptide (Adv-F/RGD)[34].

Ongoing research has reported that adenoviruses OW34, E1B55KD and HSV-TK were more effective in combination with ganciclovir than when used alone [33]. Recent studies found that systemic administration of Anti-ICAM-2 induced the complete regression of OSCC lesions[17]. However, the tumour regression is dependent on the immune system function and the induction of specific tumour toxins by the action of CD8 lymphocytes[35]. Gene transfer of IL-12 using plasmid pNGVL3-mIL12 is also being investigated. FDA approved clinical trials is under way and its to early to predict the study [36].

Excision gene therapy

This therapy aims at removal of defective oncogenes, thereby inhibiting the growth of tumour cells.6 The efficacy of akadaic acid to suppress Egr-1 (early growth response factor 1) protein expression is being studied in the OSCC setting. It is a highly toxic polyether that inhibits phosphorylation of types 1 and 2A proteins, reducing expression of Egr-1 and thereby triggering the inhibition of tumour activity. Inhibition of Egr-1 may represent a good therapeutic approach, since genes that control cell growth and cell cycle progression, including those that encode for tissue factors TGF-β1, PDGF-A and PTEN, are regulated by the expression of this protein [37].

Antisense RNA

Expression of gene can generally be inhibited by RNA, which is complementary to the DNA[6]. Antisense RNA may prevent the activity of oncogenes, including myc, fos and ras; and it can also inhibit viruses, e.g., HSV-1, HPV (Human Papillomavirus) and HTLV-1 (Human T-lymphotropic virus). Inhibition of tumor growth in Xenograft models of oral cancer with systemic administration of EGFR (Epidermal Growth Factor Receptor) antisense DNA have been observed [38].

Limitations of Gene Therapy[4]

Metagenesis- this has occurred in clinical trials for X-SCID patients in which hematopoietic stem cells were transduced with a corrective trans gene using a retro virus, which lead to the development of T cell leukemia.

Safety of vector- viruses present a variety of potential problems to the patient toxicity including immune and inflammatory responses, gene control and targeting issue.

Difficulty to treat multi gene disorder- multi gene disorders such as hypertension, alzheimer's disease, arthritis and diabetes may be difficult to treat effectively using gene therapy.

Expensive – Gene therapy is very expensive

No long term benefits because of short lived nature of gene therapy

Can induce serious side effect- it may be associated with severe inflammatory processes and coagulopathies.

Scope

Patients undergoing gene therapy are usually in a very advanced phase of the disease, and it can be difficult to distinguish whether the gene therapy or the cancer is responsible for adverse effects. The most frequently observed adverse reactions are severe inflammatory processes and coagulopathies, generally in relation to the viral vectors employed. FDA and NIH have developed a programme: The Genetic Modification Clinical Research Information System, a web base system (www.gemcris.od.nih.gov) that compiles and analyses all data related to gene therapy [30].

SUMMARY AND CONCLUSION

In the long-term, gene therapy may contribute a definitive treatment modality that offers greater effectiveness compared with current therapies and markedly reduces the high mortality associated with these lesions. At present, the use of adenoviruses to act at altered gene level and the combination of this technique with chemotherapy or immunotherapy have appeared to be the most promising approaches to the management of potentially malignant and malignant disorders of oral cavity.

The clinical application of gene therapy for treatment of oral cancer will require optimization of gene delivery. Various studies have shown promising results when gene therapy is combined with chemotherapy, radiation therapy or surgery. Such augmentative approaches, will reduce morbidity and help maintain quality of life in patients with oral squamous cell carcinoma. In future instead of supplement therapy it may even become one of the conventional cancer therapies. Further investigations are in pipeline to establish safe and effective approaches that utilize gene therapy.

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