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Gene Therapy: A Connecting Link between Medicine and Dentistry

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ABSTRACT
Genetics, coined by the British biologist William Bateson in 1909, is the study of hereditary and variation, seeking to understand the causes of the resemblances and differences in the parents and their progeny and genes. Genes are discrete units of information encoded within the DNA, which in turn is localized within chromosomes found in the nucleus of each somatic cells. Gene therapy a process essentially consists of introducing specific genetic material into target cells without producing toxic effects on surrounding tissue. In research facilities all around the world, scientists are attempting to stop diseases at their very roots. Instead of trying to find drugs to cure diseases they are trying to manipulate the genes responsible for particular disease. The dynamic therapeutic modalities of gene therapy have been advancing rapidly.

INTRODUCTION

Gene Therapy is a technique to deliver small DNA or RNA sequences to cells or tissues to correct a genetic defect or to treat a disease. The earliest applications of gene therapy were based on the principle that a disease is caused by a faulty gene (or combination of genes) and if such genes can be replaced with correct versions, the disease might be controlled, prevented or cured, either *in vivo* or *ex vivo* and not necessarily a gene that is known to cause a disease. As time passed, new technologies, techniques, strategies and ideas for transferring genes have been presented. Originally known as genetic replacement therapy during the early 1980s, gene therapy has now outgrown its original definition and is applied to all manner of protocols that involve an element of gene transfer[1].

Gene therapy is revolutionizing the dentistry by treating the oral and dental ailments. While the development of gene transfer tools is still in its infancy, these variety of applications provide an impressive spectrum of the possible applications of modern biology to dentistry[2].

Principle of gene transfer: The concept of gene therapy involves the introduction of exogenous genes into somatic cells that form the organs of the body to produce a desired therapeutic effect. The selected DNA fragment must first be cleaved using restriction endonucleases.

The next step in successful gene transfer is the preparation of the vector or vehicle used to transfer the genetic material. The vector may be viral or non viral, viral vectors majorly includes adenovirus, retrovirus and herpes simplex virus and non viral includes physical and chemical vectors. A vector must first be isolated, purified and cleaved to allow insertion of the DNA fragment. The DNA fragments then must be joined to the cleaved ends of the vector, effectively closing the molecule. This successful insertion of an exogenous DNA molecule into a vector results in a DNA chimera. These vector constructs are the basis of recombinant DNA techniques. The second step involves introduction of the construct into a cell, allowing the production of a line of genetically identical cells containing the DNA sequence introduced by the vector. This allows mass production of cells with a specifically designed gene make-up[3].

The ideal vector would have high efficiency (100% cells are transfected), high specificity and low toxicity[4]. It is highly unlikely, for the foreseeable future, that any single vector type will meet all needs for all tissues; in other words, different vectors will be needed for different clinical applications. Indeed, vector inadequacies are one of this field key shortcomings. However, some currently available vectors are quite useful for certain defined conditions, such as adenoviruses for gene therapy of head and neck cancers[5].

Of all viral vectors currently being studied, adenoviruses and retroviruses are commonly used. These viruses are attenuated to transfect genes but they cannot replicate or cause infection. Eliminating their ability to replicate through genetic manipulation of the wild type virus eliminates the pathogenecity of virus. Adenovirus-associated virus (AAV), vaccinia virus, lentivirus, herpes simplex virus and many others are currently being extensively studied in the preclinical setting[4]. Nonviral methods present certain advantages over viral methods, with simple large scale production and low host immunogenicity being just two Previously, low levels of transfection and expression of the gene held nonviral methods at a disadvantage; however, recent advances in vector technology have yielded molecules and techniques with transfection efficiencies similar to those of viruses[6].

Areas of impact

Gene therapy and bone repair: The development of effective therapies for bone regeneration is one of the most clinically important long term goals of research in the mineralized tissue field. Bone loss caused by trauma, neoplasia, reconstructive surgery, congenital defects or periodontal disease is a major worldwide dental problem. The regeneration of these bone structures poses vastly more complex problems involving specification of three-dimensional shape as well as the type of tissue formed. Yet, it would be enormously useful in the treatment of craniofacial and other bone anomalies, tooth loss, temporomandibular and other joint diseases, traumatic amputation.

In general, successful bone regeneration rests on the presence of at least four crucial elements, namely osteoinduction, differentiation of osteoblasts leading to production of osteoid matrix, osteoconduction and mechanical stimulation. Gene therapy may represent an ideal approach towards augmenting bone regeneration as it enhances the first three conditions needed for bone regeneration: Gene therapy can enhance osteoinduction via expression of growth factors, induce osteoblast differentiation and facilitate the production of osteoid matrix and utilize an osteoconductive apparatus. While first conceived as a systemic treatment for hereditary single-gene defects, localized gene therapy is well suited for bone formation because of the ability to deliver genes to a discrete site. In the case of bone regeneration, transient expression is also a desirable benefit and is readily available with existing gene transfer techniques.

Thus, gene therapy in bone regeneration has the unique ability to deliver gene products to precise anatomic locations at elevated levels for an extended duration.

The bone morphogenic proteins (BMPs) enable skeletal tissue formation during embryogenesis, growth, adulthood and healing. Probably BMPs (BMPs 2, 4 and 7) are the only growth factors who can singly induce *de novo* bone formation both *in vitro* and at heterotopic sites[7].

In studies of greater therapeutic significance from the Center for Craniofacial Regeneration at the University of Michigan, the biological activity of Ad-BMP7 was examined in two separate orthotopic regeneration models involving critical-sized defects of calvaria and long bones and a periodontal alveolar defect[8].

Although, individual BMPs can induce bone formation, there is strong evidence that these factors normally work together to induced bone formation.

The delivery of platelet derived growth factor (PDGF) for tissue engineering of periodontal wound has become an active area of interest because of its potent effect on the regeneration of hard and soft tissues. Since the growth arrest specific (gas) gene encodes the PDGF receptor, there is a downregulation of PDGF activity leading to transient biological activity and bioavailability of PDGF at the wound site.

To overcome this limitation, recently researchers at the University of Michigan have developed an *in vivo* PDGF-A gene transfer through adenovirus vector (Ad-PDGF-A). The bioactive Ad-PDGF-AA protein released induces sustained tyrosine phosphorylation and corrective downregulation of PDGF receptor, which is encoded by growth arrest specific (gas) gene.

Bone sialoprotein (BSP) is a major noncollagenous protein in bone and other mineralized tissues.

Cbfa1 is a *master gene* in osteogenesis and is involved in BSP gene expression, which controls the cell differentiation during bone repair and regeneration.

By the *in vivo* delivery of a BSP gene into an osseous defect, it has been shown to regenerate periodontal alveolar bone[9].

Gene therapy in salivary gland disorders: Salivary gland destruction occurs as a result of various pathological conditions, such as radiation therapy for head and neck cancer and Sjogrens syndrome (SS). Accordingly, the development of a novel treatment to restore or regenerate damaged salivary gland tissue was much awaited till the development of gene therapy[10]. Salivary glands are excellent target sites for gene transfer. They are capable of producing large amounts of proteins and are also encapsulated, a circumstance likely to minimize the undesirable access of administered vectors and transgenes to other tissues[11]. The anatomical structure of the salivary gland, which resembles the many branches and the trunk of a tree, explains that the apical pole of each glandular cell is accessible for gene delivery by a minimally invasive procedure.

The principal lesion in SS is lymphocytic infiltration in target tissues. Potential target genes in gene therapy for SS-damaged hyposalivation include inflammatory mediators, cytokine inhibitors, apoptotic molecules, cell to cell interaction or intracellular molecules

Gene therapy in oral cancer: Squamous cell cancer of the head and neck (SCCHN) is the sixth most common cancer worldwide and includes cancer of the oral cavity, pharynx, larynx and paranasal sinuses. In contrast to cancer in other parts of the body, head and neck cancer is an attractive target for local gene therapy because of its anatomical location. This allows delivery of vectors directly to the desired site with only a small risk of systemic toxicity. Several strategies have been developed for cancer gene therapy, including:

- Immunogenic therapy, which involves modulation of immune responses through the transfer of cytokines, immune accessory molecules or tumor antigens
- Antiangiogenic therapy, which involves the introduction of genes with antiangiogenic properties in a variety of tumor cells
- Oncolytic virus therapy, which selectively kills tumor cells but not normal cells
- Gene replacement therapy, which involves the introduction of tumor suppressor genes, such as p53, in cancer cells
- Suicide gene therapy, which is used to transduce cancer cells with a gene construct that is able to convert a prodrug into an active drug that is toxic for target cells[12]. These approaches may converge and can often be used in combination to amplify potential therapeutic effects

The incidence of p53 mutations in head and neck cancer is believed to be higher in recurrent disease. Replacing a mutated p53 gene with a wild-type (normal) p53 gene is a potential approach to head and neck cancer treatment. This approach is limited by the lack of mutated p53 in many tumors and also by the current limitations of vector technology in delivering the gene. In a study of 17 patients with advanced recurrent or refractory unresectable head and neck cancer, treatment with delivery of the p53 gene using an adenoviral vector found only two patients with tumor regression of more than 50%. Loss of p16 expression is secondary to allelic loss of the 9p21 locus and mutation and/or hypermethylation of the gene.

Inactivation of p16 is believed to be one of the first steps in head and neck cancer carcinogenesis and may therefore be an ideal target for gene replacement therapy.Re-expression of p16 in experimental models using viral constructs has the ability to reverse tumor growth and induce apoptosis[13]. Conditionally replicating adenoviruses (CRAds) represent a novel class of anticancer agent. These viruses are genetically modified to selectively replicate in tumor cells and to destroy these cells by inducing their lysis[14]. Over the past decade, several oncolytic viruses have been tested in humans and although the safety results are encouraging, efficacy as single agents was limited. Possible hurdles include attenuation of the virus caused by genetic engineering of the virus that renders cancer selectivity, host immune responses and lack of understanding of tumor microenvironment. However, H101, an oncolytic adenovirus similar to Onyx-015 (E1B-55K/E3B-deleted), was recently approved by the Chinese government to be used in conjunction with radiation therapy for the treatment of head and neck cancers. This is the first oncolytic virus product approved by a governmental agency for human use[15].

Further optimization of vectors is essential for improving the clinical efficacy of cancer gene therapy.

Gene therapy in 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. The use of gene transfer in place of drug delivery to achieve the continuous release of short lived bioactive peptides in or near the spinal dorsal horn underlies the most common strategies for gene therapy of pain. There are two principal models. The first involves intrathecal injection of vectors derived from adenovirus, AAV or lipid encapsulated plasmids. Both have demonstrated robust antiallodynic and antihyperalgesic effects with prolonged effects shown after two injections of a plasmid coding interleukin-10. The cells transduced by vectors injected into the cerebrospinal fluid are known to include resident meningeal cells lining the intrathecal space as well as neurons and glia in spinal parenchyma. In the second approach, neurons of the dorsal root ganglia (DRG) are transduced by injection of herpes simplex virus based vectors into the skin. These naturally neurotropic vectors are carried by retrograde axonal transport from the skin to the neuronal perikaryon of the DRG. Here, they effect production of inhibitory neurotransmitters 6 or antiinflammatory peptides to reduce pain in several different chronic pain models. Gene therapy in Keratinocytes

Epidermal and oral keratinocytes are potential vehicles for gene therapy. Several features of these tissues can be utilized to achieve delivery of therapeutic gene products for local or systemic delivery.

These qualities include:

- The presence of stem cells
- The cell-, strata- and site-specific regulation of keratinocyte gene expression
- Tissue accessibility
- Secretory capacity

Such features can be exploited by the use of gene therapy strategies to facilitate:

- Identification, enrichment and targeting of stem cells to ensure the continued presence of the transferred gene
- High-Level and persistent transgene expression using keratinocyte-specific promoters
- Tissue Access needed for culture and grafting for *ex vivo* therapy and direct *in vivo* gene transfer
- Secretion of transgene product for local or systemic delivery
- Monitoring of genetically modified tissue and removal if treatment termination is required. Optimal gene therapy strategies are being tested in a variety of tissues to treat the dominant and recessive genetic disorders

DNA vaccination: 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. The ability to induce an immune response to a protein antigen by administration of plasmid DNA encoding the antigen has been successfully demonstrated in animal models. DNA vaccines consist of a eukaryotic expression vector containing a target gene of interest. While DNA vaccination with a single bacterial gene is ostensibly still a subunit approach to vaccination, it is particularly attractive compared to administration of a preformed protein antigen because the immunogen of interest is actively synthesized *in vivo* in transfected cells[16]. Many studies had reported that mucosal delivery of DNA in liposome and other materials enhanced the mucosal immunity. It has been reported that the plasmid pCIA-P encoding pac gene of *S.mutans* could induce protective anticaries immune responses in rats by targeted salivary gland immunization[17].

Human periodontitis is thought to be initiated by a principal organism called *P. gingivalis*. Two separate rgp-encoding genes (rgpA and rgpB) are located on the chromosome of *P. gingivalis*. rgpA may play a central role in the pathogenesis of periodontal disease via production of pathophysiologically significant proteins. A study demonstrated that immunization of mice with the rgpA DNA vaccine protects against challenge with invasive *P. gingivalis* strain W50 in the mouse lesion model. A study showed that delivery of the cDNA for the *P. gingivalis* fimbrial protein into murine salivary glands led to the production of secretory immunoglobulin A specific for this microbial protein. This approach could be used to immunize humans against other oral microbes, such as mutans streptococcus.

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.

Molecular targeted therapies in head and neck cancer: Squamous cell carcinoma of the head and neck (SCCHN) represents the eighth leading cause of cancer worldwide. Despite recent advances in surgery and radiotherapy, overall cure is achieved in less than 50% of patients. In contrast to many other cancers, distant metastases are rarely present at diagnosis but due to better local control, the incidence of systemic spread is rapidly increasing. Those with recurrent or metastatic disease have a poor prognosis, with median survival rates of 6-10 months[18].

Systemic chemotherapy remains the only effective treatment option but it is associated with significant toxicity rates in HNSCC patients, who usually have a high prevalence of co-morbidities and problem free lifestyle[19].

New agents that specifically target cellular pathways associated with carcinogenesis are promising candidates, because they are already successfully used in other hematological malignancies as well as in solid tumours, such as colorectal or lung cancer[20].

The role of EGF-R signalling in HNSCC: The EGF-R is a member of the human epidermal receptor (HER)/Erb-B family, a group of tyrosine kinases that transduce extracellular signals to intracellular responses influencing cell proliferation, apoptosis, angiogenesis and the capacity of tumour cells to metastasize It has been shown that EGF-R and TGF-a, one of the seven known ligands of EGF-R, are overexpressed in many solid tumours, including colorectal cancer, NSCLC and HNSCC[21]. Furthermore, EGF-R-overexpression as well as increased m-RNA levels of TGF-a I tumours are usually associated with poorer responses to radiotherapy and have been shown to be strong predictors of decreased disease-free survival[22]. These observations are the rationale for the development of EGF-R-targeted therapies, which are intended to interrupt EGF-R-mediated pathways Among EGF-R-targeting therapies, there are two large categories of molecules: Monoclonal antibodies, which recognize the ligand-binding domain and interfere with receptor activation and tyrosine kinase inhibitors which bind to the cytoplasmatic region and influence with downstream signalling events.

Anti-EGF-R antibodies: Cetuximab is a chimeric human/murine monoclonal antibody of the IgG1 isotype that binds to the EGF-R with a higher affinity than its endogenous ligands, preventing dimerization, internalisation and autophosphorylation. Preclinical studies show at least three different mechanisms by which cetuximab affects tumour cells. First, it enhances tumour-cell apoptosis and inhibits proliferations well as invasiveness by blocking the tyrosine-kinase mediated pathways. Second, antibody-dependent cell mediated toxicity, which is associated specifically with theIgG1 isotype, contributes to the anticancer activity. Finally, cetuximab may block the nuclear import of EGF-R, preventing activation of the DNA repair mechanism that protects cells from radiation- or chemotherapy-induced DNAdamage[23-25].

Cetuximab in metastatic HNSCC: In previously untreated patients with metastatic HNSCC, cisplatin-based chemotherapy is considered standard. This approach is now challenged by the recently published results of the EXTREME study (Erbituxin First-line Treatment of Recurrent or Metastatic Head and Neck Cancer). In this controlled randomized phase III trial, 442 patients who were not amenable tolocal therapy and had not received any systemic treatment received either cisplatin or carboplatin, together with 5-fluorouracil or a combination of this chemotherapy with cetuximab.

EGF-R-targeted tyrosine-kinase inhibitors TKIs bind intracellularly to EGF-R tyrosine-kinase and block downstream signalling pathways. Gefitinib and erlotinib, both administered orally once a day, are the two most advanced TKIs and are both approved for certain indications in non-small cell lung cancer. They have been evaluated in phase I/II trials as monotherapies in recurrent or metastatic HNSCC with response rates of 4-10%.

EGF-R and HER-2 combined targeted tyrosine-kinase inhibitors: HER-2 has also been found to be expressed in a significant proportion of EGF-R-positive HNSCCs. Since EGFR and HER-2 heterodimerize to form

functional signaling complexes, tyrosine-kinase inhibitors with dual specificity against HER-2 and EGF-R, such as lapatinib,have been investigated in phase I and II studies. In one of these studies lapatinib, which is already approved for breast cancer treatment, showed disease stabilization rates of about 20% in patients pretreated with anti-EGFR compounds and therefore its efficacy in the adjuvant setting is currently being explored in ongoing phase III studies[26].

The role of angiogenesis in HNSCC: Similar to other solid tumours, angiogenesis plays an important role in the pathogenesis of HNSCC. Vascular endothelial growth factors (VEGF) and its receptors are expressed in most cases of HNSCC and multiple preclinical studies have shown that these markers area ssociated with tumour progression, changes in microvessel density and development of lymph node metastasis.

In addition, increased levels of VEGF in serum of patients with HNSCC appear to induce tumour growth, metastasis and treatment failure[27].

In HNSCC, bevacicumab shows little single agent activity but a small phaseI/II study in combination with erlotinib in metastatic or incurable recurrent disease showed an overall response rate of about 15% and a median survival of 7.1 months. In general the regimen was well tolerated, with rash, diarrohea and fatigue as the predominant side effects but as in other entities, a small but significantly increased risk of bevacicumab-associated bleeding events have been reported in addition to other, more easily manageable side effects, such as hypertension and fluid retention, in this trial[28]. In several ongoing trials bevacicumab is currently being explored in combination with chemotherapy, radiation therapy, or EGFR inhibitors,but so far no clinical data are available to recommend the use of bevacicumab in the clinical routine.

Other potential targets: Src kinases are involved in the regulation of a variety of normal cellular signal transduction pathways and they influence cell proliferation, survival, angiogenesis, migration and adhesion. In general, levels of Src expression or activation in epithelial tumours correlate with disease progression. It is important that Src activation results in potentiation of EGF-R-mediated tumour growth by stimulating the same downstream pathways like FAK, STAT and PI3K[29]. In fact, recently published in vitro experiments show that Src family kinases are highly activated in cetuximab-resistant cells and that they enhance EGF-R activation despite the cetuximab-bound receptor. In these experiments inhibition of Src kinases in originally cetuximab-resistant cell lines resulted in are gaining of sensitivity against cetuximab, indicating a close interaction between SRC and EGF-R regarding the processes causing cetuximab resistance in tumour cells[30-31].

CONCLUSION

The role of gene therapy in dentistry will continue to evolve in the on coming years. There are many confounding parameters in establishing a genetic link for manifestation of dental diseases. Of many factors which act as risk, modifying or predisposing factors some may be heritable, such as diabetes and propensity for tobacco use, where as others may simply cluster within families or cultures, such as oral hygiene practices and other health beliefs, which will impact on disease process. Although, the problems of introducing genetic technologies are still substantial; it is anticipated that the future of dentistry will be "GENETIC"; genetic prevention , genetic diagnosis and genetic therapy.

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