A New Strategy For The Genetic Therapy Of Oral Cancer: An Update

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ABSTRACT

Following advances in treatment approaches such as radiation therapy, surgery, and chemotherapy, the quality of life of people with cancer affecting oral cavity throughout the past several centuries has not greatly benefited. There are few available treatments for oral malignancies that are recurring or resistant. Clinical studies are being conducted right now to examine gene therapy for oral cancer. By giving target cells a fresh genome without harming surrounding organs, gene therapy fights cancer. Our present article highlights the approaches adopted in gene therapy for oral cancer

KEYWORDS: Gene Therapy; Oral Cancer; Gene; Treatment

INTRODUCTION

Most of the estimated 42,800 new instances of head and neck cancer each year are believed to be caused by oral cavity and oral pharyngeal tumor's, which are among the most deadly of all human malignancies. The usual course of treatment is radiation, sometimes chemotherapy, and extensive deforming surgery. Along with the severe aesthetic deformity, surgical excision typically leaves patients with major functional limitations in their ability to speak, swallow, and use their upper extremities. Additionally, radiotherapy causes significant morbidity such as discomfort, xerostomia, mucosal atrophy, soft tissue fibrosis, and radio-necrosis of the mandible and larynx. The use of chemotherapy regimens is not without risk; common side effects include toxicity to digestive tract, bone marrow, eyes, and kidneys. Because no clinical trials have yet demonstrated improved survival, the efficacy of adjuvant or induction chemotherapy in head and neck cancer remains disputed. Despite years of research, the prognosis for individuals with head and neck cancer remains grim, with 2-year survival rates rarely exceeding 30% in those with severe stage III and IV disease. Due to the dismal outcomes of standard therapy, gene therapy has become a new emphasis in treatment plans.[1]

THE USE OF GENES

Gene transfer with the goal of curing human illness is referred to as gene therapy. It employs genes as a kind of medication and is made to introduce genetic material into cells to make up for defective genes or to promote the production of a helpful protein. It is a new area of biomedicine. While protecting healthy tissues, it has the ability to attack cancer cells. From single gene disorders to multi-gene disorders, it may be utilized to treat a broad variety of diseases. In the realm of dentistry, it is also used for cancer, immunological illnesses, healing of bones, sensation of pain, and DNA vaccination (caries and periodontal diseases). [2]

A GENETIC THERAPY HISTORIANA

The fundamental assessments for gene therapy were developed by J Lederberg and E Tatum. Thymidine kinase (TK) gene was successfully introduced into human cells by Michael et al. in 1977, gene therapy technology advanced significantly. On September 14, 1990, The National Institutes of Health team gave Ashanthi De Silva, a four-year-old child, her own T cells that had been altered with a retroviral vector carrying a normal ADA gene. Both severe combined immunodeficiency and ADA deficiency have been identified in Ashanthi De Silva.

ORAL CANCER'S MOLECULAR DIAGNOSTIC

The inactivation of cellular negative regulators is a critical stage in the changing potential malignant into a cancerous tissue. Tumour suppressor genes are found in the human genome. When the p53 tumour suppressor gene is mutated, the protein is either structurally changed or altogether deleted. Restoration of p53 in oral cancer lines and tumours generated in animal models has shown that p53 is functionally inactive in oral tumours. Proto-oncogene mutations and changes in the genes that encode cell adhesion molecules are two additional variables besides p53 alterations that influence the growth of cancer. Oral cancer has also been linked to certain viruses. [3]

GENE THERAPY IDEAS

Gene therapy aims to replace existing genetic information in target (cancerous) cells while sparing neighboring healthy cells and tissue from damage. It is described as the "genetic modification of patient cells to combat a disease." Both the addition of newer genomes and the change of already existing genomic information are included in the treatment of genes.

Following steps that make up the most popular gene therapy process in use today are as follows:

1. The treatment's target gene's identification, isolation, and amplification;
2. Taking tissue cells from the patient to be treated and cultivating them in a lab;
3. Therapeutic gene is sent into these cells via a vector, which carries a gene with a promotional sequence to promote its expression and a marker to recognise the cells it has been inserted into;
4. Transcription;
5. Translation According to the notion, the sickness will vanish once the gene performs its typical physiological activities. [4]

THE APPLICATION OF GENETIC THERAPY TO TREAT ORAL CANCER

A promising method for creating more potent anticancer therapies and minimizing treatment-related harm is to specifically target the genetic abnormalities responsible for cancer growth and carcinogenesis. It is possible to destroy cancer cells selectively by expressing any one of a number of known genes at the proper location and dosage. Gene therapy has not yet been shown to be suitable for intravenous administration in tumour patients, despite the fact that intravenous injection is hypothetically preferred to treat metastatic tumours. Given how easy it is to administer gene therapy to both primary and recurring lesions in head and neck cancer, this disease is a top target for the treatment. [5]

A RANGE OF GENE THERAPIES

Somatogene Treatment

Somatic gene therapy is only available to the patient receiving it. In somatic gene therapy, gene expression is changed in cells for the patient's benefit but is not passed down to offspring. The development of somatic cell gene therapy is still in its infancy. Three subcategories of somatic gene therapy are possible:

A. In vivo
   It entails extracting cells from the body, genetically modifying them, injecting a medicinal Genome carrier into them, and reinstalling the transformed cells into the host. With skin and blood cells, this has generally been an option up until now.
B. In-situ.
   Putting the therapeutic DNA-carrying vector right into the organ or tissue where the gene will be active. On the lungs and airways of individuals with cystic fibrosis and other cancer kinds, this has been studied.
C. In vivo
   In this situation, a therapeutic DNA-carrying vector might be administered intravenously and then taken up by target cells.

GENE THERAPY BASED ON GERM LINES

A hereditary genetic alteration will result from germ line-based gene therapy. In germ line gene therapy, in order to prevent a genetic defect from being handed down to succeeding generations, embryonic cells (sperms and ova) are genetically modified. [6] Because shallow lesions typically allow for the direct injection of genetic material, cancer of the oral cavity is still being treated using the in vivo technique. [7]

PRINCIPLES OF GENE THERAPY

Select the desired gene, obtain a healthy copy of it using a restrictive endonuclease enzyme, and then clone the therapeutic gene into a vector that will act as the target gene's delivery mechanism. The remedial gene is delivered to the patient's target using a carrier gene. The remedial gene infects the target cells via the vector. The

healing gene creates useful proteins that aid in the cell's return to normal function. [8]

DESCRIPTION OF GENE TRANSFER GENERAL PRINCIPLES

In order to achieve the desired therapeutic outcome, the idea behind gene therapy is inserting foreign genes into somatic cells that make up the body's organs. Utilizing restriction endonucleases, the chosen DNA fragment is first cut. After that, the vector or vehicle for the genetic material transfer is created. In order to insert the DNA fragment, the vector must first be separated, purified, and cleaved. The vector's cleaved ends must next be linked with the DNA fragments to effectively close the molecule. A DNA chimaera is created as a result of this exogenous DNA molecule's successful insertion into a vector. The foundation of recombinant DNA technology is these vector constructions. A line of genetically identical cells with the DNA sequence given by the vector are created when the construct is introduced into a cell. This enables the mass synthesis of cells with a certain genetic make-up. It is possible to inject the vector systemically or locally into a particular area of the body. The vector is then utilised by particular target cells after being picked up. Instead, the vector can be exposed to representative patient cells that have been removed from the body in a lab setting. The patient receives the cells, which now contain the vectors—for the purpose of treatment. The patient's target is where the therapeutic gene is vectored into. The therapeutic gene infects the target cells via the vector. The therapeutic gene generates functioning proteins, which help the cell advance to a normal state. [9]

VECTORS AND REQUIREMENTS OF VECTORS

Genetic matter can be transmitted using a vector, which is the tool used to convey the desired gene. Each target cell would get a precise dosage of genetic information from the optimal vector, enabling the gene product to be produced without causing any harm. [10]

The perfect gene transfer vector ought to possess the following qualities:

- It ought to be administered with the least amount of intrusion;
- It ought to be targeted to particular cells;
- It ought to express the transgene product (protein) at a therapeutic level;
- It ought to have longevity of expression under strict regulation;
- It ought to have high efficiency;
- It ought to show essentially no toxicity. [11]

TYPES OF VECTORS

Viral and non-viral gene therapy vectors fall into different categories. The most often used viruses as vectors are adenovirus, retrovirus, and herpes simplex virus (HSV). Adenovirus is one of them that is frequently employed since it is easy to culture and has a reduced pathogenicity. These viruses are weakened to transfect genes, but because they cannot reproduce inside of cells, they do not infect cells. Transduction is the name of the viral vector-mediated gene transfer mechanism.

The adeno-associated virus (AAV) is a small virus that only integrates about half as much foreign DNA as other viruses. The functional gene can be quickly transferred from this virus to chromosome 19 at a specific site. The main drawback of this vector is that, when used in vivo, it causes the innate and humoral immune pathways of the recipient to become activated.

Only dividing cells can be infected by retroviruses. These vectors will produce stable function and stable expression of germ cell lines as they permanently integrate the foreign DNA into the chromosomes of the host cell. Genetic mutation, however, could happen if the interchange of genetic material is not controlled. These vectors require mitotic cells for transduction. HSV double-stranded viruses specifically target particular cells, such as neurons.

Physical and chemical vectors are additional categories for non-viral vectors. Transfection is the term used to describe employing vectors that are not viral to transfer genetic information. Electrophoration, microinjection, and the usage of ballistic particles are examples of physical vectors. Lipids, protein complexes, and calcium particles are examples of chemical vectors.

Electrophoration: Through pores that are produced by the flow of electric current, DNA is transported into the desired cells using the electrophoration technique.

Microinjection: DNA is injected into a single cell using the microinjection technique.

Ballistic particle use: The genome of a plasmid is covered with particles made of gold or tungsten. A high-voltage electrical spark or helium discharge produces an accelerated force that propels the particles into the tissue.

Calcium vectors: In both in laboratory and in live settings, DNA-doped calcium phosphate nanoparticles of ultra-low size and highly mono-dispersed size can be used safely to convey the DNA that has been encapsulated.

Lipid vectors: This method involves the introduction of plasmid DNA and liposomes, which are synthetic lipid spheres with an aqueous centre. The liposomes eventually

fuse with the cell membrane, allowing the plasmid DNA to enter the cytoplasm.

Protein complex: On the cell surface, there are protein molecules that function as cell surface receptors and can serve as DNA attachment sites. As a result, ligands for DNA will enter cells when they are attached to these receptors.

Non-viral vectors have certain benefits, including easy DNA injection techniques, widespread vector manufacturing, and minimal host immunogenicity

Disadvantages of non-viral vectors: Only particular tissues may be utilised for the operation, and a significant amount of DNA is needed.

Stem cells can be employed for gene transfer in addition to viral vectors. They are modified in the lab to have the capacity to receive additional genes, which changes how they operate. They are then injected into the targeted regions. [12]

STEPS IN GENE THERAPY

Gene therapies that cause cancer cells to die include:

- Gene addition therapy
- Gene excision therapy
- Antisense ribonucleic acid (RNA) therapy
- RNA interference (RNAi)-based gene therapy
- The delivery of drug resistance genes into healthy tissues to fend off chemotherapy.
- Immunotherapy,
- suicide genetic treatment, and
- oncolytic virus-based gene therapy are all examples of complementary and alternative medicine.

GENE ADDITION THERAPY

By introducing tumour suppressor genes that silence cancer-causing cells, it is a technique for controlling tumour growth. In contrast to normal cells, cancer cells show disturbed cell cycle and death as a result of mutation. Oral cancer has been linked to genetic changes, such as p53, retinoblastoma gene (RB1), p16, and p21 mutations. P53 is commonly changed during oral carcinogenesis; it was also mutated in cancers involving precancerous lesions and diseases involving the epidermal growth factor. These reasons have led to the development of numerous viral vectors, mainly adenoviral vectors, and made p53 the most often used tumour suppressor gene in gene therapy. Currently, an intramucosal injection of the adenovirus vector Ad5CMV-p53 followed by oral administration is being used in a phase III trial.The same treatment regimen, which is repeated every 28 days, has demonstrated the ability to slow the spread of precancerous lesions without having any hazardous side effects. For their apoptotic and anti-tumor effects on interleukin 2 and mIL-12 (murine interleukin), Rb (retinoblastoma gene), p27, and mda-7 (melanoma differentiation-associated gene-7) can also be used in gene addition therapy.

THERAPEUTICAL GENE EXCISION

Damaged oncogenes are eliminated by a procedure known as gene excision therapy to halt the growth of cancer cells. Okadaic acid, a very toxic polyether, is used to stop the development of tumours by reducing the expression of Egr-1 (early growth response factor 1). Inhibition of Egr-1 may be a successful therapeutic approach since it controls the expression of genes that impact cell growth and cell cycle development, including those that encode for the tissue factors TGF-â1, PDGF-A, and PTEN.[13]

RNA THERAPY USING ANTISENSE RIBONUCLEIC ACID

The complementary RNA to the DNA strands that express that particular gene is blocked by the antisense RNA, inhibiting the tumor's expansion. The expression of these oncogenes (MYC, FOS, and RAS) may be suppressed, which would prevent cancer growth by altering the phenotype. In preclinical studies, antisense sequences regulated by six short RNA promoters were employed to demonstrate a powerful anti-tumor effect with minimal side effects. The difficulty of delivering enough antisense molecules to stop cancer growth hinders the use of this technique. Strong promoters are currently being developed in order to solve this problem.

GENE THERAPY BASED ON RNA INTERFERENCE (RNAI)

Small interfering RNA (siRNA) plays a major role in controlling messenger RNA degradation. Two techniques are used in this kind of gene therapy:Using viral or plasmid vectors, short hairpin RNA precursors can be directly delivered to target cells.Self-complementary recombinant adeno-associated virus (sc AAV), which transported siRNA into multidrug-resistant human breast and oral cancer cells, successfully reduced multidrug resistance-1 (MDR-1) gene expression. This resulted in a rapid, striking, and persistent reduction in the expression of the P-glycoprotein multidrug transporter as well as a considerable reversion of the drug resistance phenotype.

TRANSFER OF DRUG RESISTANCE GENES FOR CHEMOTHERAPY PROTECTION TO NORMAL TISSUE

Drug resistance genes are those that prevent healthy tissues from deteriorating. The drug resistance gene MDR-1 is present in humans. This collection of genes also include the gene for bacterial nitroreductase and the

methotrexate-resistant dihydrofolate reductase mutants.[14]

IMMUNOTHERAPY

Natural killer cells, lymphocytes, cytokines, and other components of the immune system are frequently deficient in or malfunctioning in oral cancer patients. The method enhances an individual's defence system's capability to combat cancer. [15]

SUICIDE GENETIC TREATMENT:

In this Genetic treatment host is exposed to the genes that encourage the production of cell-toxic byproducts. Suicide genes allow for creation of enzymes that can change not hazardous drugs into ones that are hazardous. The administration of ganciclovir coupled with the delivery of the HSV thymidine kinase gene via an adenovirus vector may be beneficial for the treatment of oral tumours. One of the primary issues with suicide gene transfer is the inadequate vector dispersion within the tumour [16].

THERMAL GENESIS USING ONCOLYTIC VIRUSES

In this therapy, cancer cells are multiplied and killed using a genetically modified virus. For more advanced tumours than conventional medicines, adenovirus-mediated gene therapy is used.

ADVANTAGES

1. A functioning gene can take the place of a damaged gene.
2. Gene therapy helps guard against the possibly hazardous effects on the body that other therapies may have.
3. Numerous therapy' costs are reduced, and the patient's quality of life is improved for a longer period of time. [17]

LIMITATION AND PROSPECTS

There are numerous obstacles to overcome in cancer gene therapy, despite some encouraging results. In clinical trials, only a small subset of therapeutic genes may be employed. In vivo, vectors are ineffective. Although adenovirus has a well-documented high transfection effectiveness in vitro, it is still unclear if adenoviral vectors are beneficial in vivo in solid tumour models. [18]

FUTURE SCOPE

There is still significant work to be done to solve some of the issues that may prevent future treatments from being effective, even though recent developments in therapeutic genetics have all led to higher tumour fighting abilities. One of them is viral vector modification to lower immunogenicity and toxicity while increasing the transduction efficacy of nonviral vectors. Another method is to use tissue- or cell-specific promoters to boost the vector's specificity.

A research of gene therapy combinations utilizing suicide gene and cytokine immunogene therapy, or tumour suppressor gene and oncogene-inactivating constructs; and a reduction in the unfavorable effects brought on by using retrovirus vectors for human gene transfer.

Future cancer gene therapy patents will mostly focus on addressing these problems [19].

CONCLUSION

It is reasonable to anticipate rapid clinical advancements in cancer gene therapy. By choosing the most likely successful therapeutic combinations based on both the genetic analysis of cancers and preclinical investigations seeking to cure specific biological flaws, progress will be made. There is little question that a new age in cancer treatment is only getting started. [20]

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