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Novel Therapy for Oral Cancer - Gene Therapy an Update

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Authors' contributions

This work was carried out in collaboration between all authors. Author NAK prepared of body of manuscript. Author AA prepared of manuscript and search of reference article. Author RS managed the referencing and conclusion of article. All authors read and approved the final manuscript.

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ABSTRACT

Gene therapy provides modern medicine with new perspectives and had great potential as a novel therapeutic modality. Progress in molecular biology, especially molecular medicine is now changing the basics of genetic disease. This technology takes advantage of our understanding of cancer at the molecular level. It has been exploited to develop new strategies for killing cells selectively or arresting their growth. This is new technique, being developed which offers incredible pledge for the upcoming therapeutic modality in oral cancer treatment. The aim of this paper is to review delivery routes, vector design, therapeutic applications and possible obstacles faced by gene therapist.

Keywords: Gene therapy; oral cancer; oncogenesis; vector.

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1. INTRODUCTION

Genes are specific bases that encode instruction to make proteins [1,2]. Gene therapy (Fig. 1) can be defined as gene transfer for the purpose of treating human disease [3]. It uses genes as medicine [4] and is designed to introduce genetic material into cells to compensate for abnormal genes or to enable the making of a beneficial protein [1]. It is an emerging field of biomedicine [5]. It has the potential to target cancer cells while sparing normal tissues [3]. It can be used to treat wide range of diseases ranging from single gene disorder to multi-gene disorder [6]. In the dental field, it is also applied in bone repair, autoimmune diseases, pain [7-9], DNA vaccination (caries and periodontal diseases) and cancer [6,7]. Minor salivary glands and keratinocyte present in the oral mucosa are the excellent target sites for gene therapy since it can be readily accomplished with minimal invasive manner. This makes dental surgeon suitable as gene therapist [6].

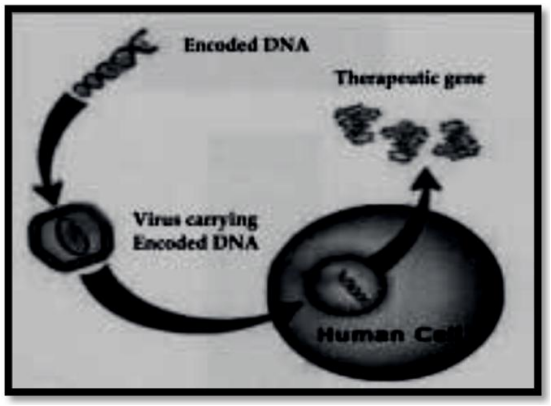


Fig. 1. Gene therapy

Oral Squamous Cell Carcinoma (OSCC) is one of the most common malignancy seen globally [5] and it ranks 6th amongst all cancer worldwide [8]. OSCC is a disease in which the genes that control the cell growth and apoptosis are mutated and the cells invade into deeper tissues and metastatize [10]. The current treatment strategies for OSCC include a combination of surgery, radiation therapy and chemotherapy [11]. Recurrence develops in approximately one – third of the patients despite of definitive treatment [12]. Inspite of all research in the field, the five year survival rate has not shown any improvement over the past 4-5 decades [13]. Oral cancer accounts for 2% of cancer deaths in males and 1% of cancer deaths in female [14].

1.1 Sources and Selection Criteria

We searched Medline and Google, using the terms “gene therapy” and “gene therapy in oral cancer.” Articles from PUBMED indexed/Medline journal between the year of 1996 and 2014 are opt for this review.

1.2 History of Gene Therapy

Gene therapy fundamentals were laid by Joshua Lederberg and Edward Tatum [12]. Historically, the discovery of recombinant DNA technology in the 1970’s provided the tools to efficiently develop gene therapy. In 1977 a large step took place in the field of gene therapy by Michael et al and he was succeeded in transferring a gene thymidine kinase (TK) into mammalian cells [15]. Rosenberg et al in 1989 started with first human gene therapy trials [16]. In 1990, the first successful treatment of a human disease by *ex vivo* gene replacement therapy was the treatment of X-linked Severe Combined Immunodeficiency Syndrome (SCID) [17,18]. Till 1990’s there was no gene research lab in India. In 1998 the first centre dedicated to gene therapy research is Advanced Centre for Treatment, Research and Education for Cancer (ACTREC, Mumbai) by Rita Mulherkar’s group doing studies related to the treatment of head and neck cancer using viral vectors. The most recent research lab in India started in Bengaluru in 2013 and presently 10 labs (Graph 1) are working on gene therapy in India, most of them are working on gene defects not related to head and cancer except one present in Mumbai (ACTREC) [15].

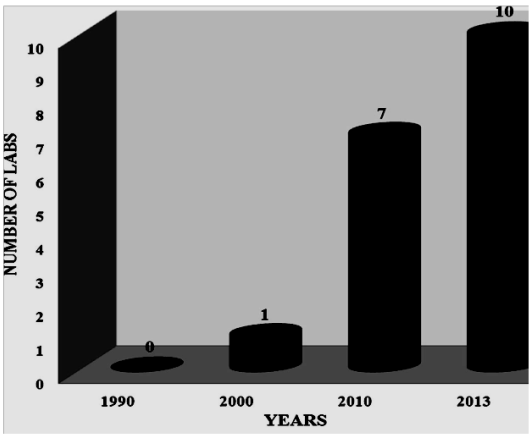
Genetic basis of Oral cancer Oncogenesis involves a series of genetic steps and also epigenetic. The genetic mechanism behind cancer is the over-expression of oncogenes and / or the silencing of tumor suppressor genes (TSGs). Cell cycle control is disturbed particularly by over-expression or over-activity (amplification) of oncogenes which drive cell proliferation.

1.3 Oncogenes

The over-expression of oncogenes -such as the epidermal growth factor receptor (EGFR) gene – can promote growth, survival, and spread of cells - leading to the development of cancer.

1.4 Tumor Suppressor Genes

Tumor Suppressor Genes (TSGs) are genes that normally function in growth control - by regulating the cell cycle, programmed cell death (apoptosis), cell adhesion and DNA repair. Deletions or mutations or silencing occur via damage by oxidation and chemical free radicals and any of these changes can lead to cancer. Rate of mutation increased by various cancer risk factors; mainly exogenous factors: Tobacco, alcohol, chemicals, radiation (e.g. sunlight, ionizing), infections, diet (some aspects can harm and some protect) or immuno-incompetence are relevant [19].



Graph 1. Number of Gene therapy labs in India

Gene mutations have been detected in oral SCC in chromosomes 3p, 4q, 6p, 8p, 9p, 11q, 13q [retinoblastoma (Rb) tumor suppressor gene], 14q, 17p (p53 tumor suppressor gene), 18q [deleted in colon cancer (DCC) tumor suppressor gene] and 21q.

Normal oral keratinocyte division is stimulated by epidermal growth factor (EGF) binding the EGF receptor (EGFR) which activates ras. Active ras triggers the kinase cascade (RAF, MEK, MAPK) resulting in increased levels of c-myc in the nucleus. c-myc stimulates the transcription of cyclin D which activates cyclin-dependent kinase (CDK). Active CDK catalyzes the phosphorylation of the retinoblastoma tumour suppressor protein (pRb). Phosphorylated pRb releases the E2F transcription factors which are required for the transcription of DNA replication proteins including proliferating cell nuclear antigen (PCNA). DNA damage in oral keratinocytes is detected by p53. As a result, there is an increase in the level of p53 which stimulates the transcription of p21, a CDK inhibitor which blocks the phosphorylation of pRb. p21 also binds and deactivates PCNA. p53 stimulates the transcription of Bax which blocks the activity of bcl-2. Caspase 3 activity is unchecked and apoptotic cell death proceeds. If the p53 gene is mutated, the protection offered by the p53 tumor suppressor protein against DNA damage (for example, *ras* oncogene mutation) is lost [20].

Gene therapy employs two approaches i.e. either transgene is introduced into the target cell directly (*In vivo*) or target gene is taken out from the body and transgene is introduced into the target cell and is re-implanted into human body (*Ex vivo*) [6].

Usually *In vivo* approach is not effective. In *Ex vivo* approach vector (carrying the therapeutic gene) is inserted into the target cells either intravenously or injected directly into specific tissue in the body, where they are utilized by target cells [1,2,4,6].

Primarily, the main concept behind gene therapy include gene addition, removal of a harmful gene, and control of gene therapy.

When therapeutic gene are introduced into somatic cells (body cells) only, and the recipient's genome is changed, but the change is not passed to next generation then are called as Somatic gene therapy and other type of it include gene introduction into the germ cell (sperm and egg) thus permanently altering the genes inherited by future generations and passed on to the offspring. This type of therapy is prohibited in many countries due to ethical and technical concerns [21].

Vector is defined as the vehicle that is used to deliver the gene of interest. Vector (Table 1) delivers the therapeutic gene into patients target cell [5]. One of the most commonly used vector to carry modified genes into cells is the virus. Viruses (Fig. 2) are natural infectious agents for transferring genetic information [22] i.e. they attach to host cell and transfer their viral genetic material into it. They then take over the cell and use the cell components to make copies of the virus and other type is non-viral vector. Viral vector has to be non-immunogenic, stable, easily reproducible, increased longevity of expression [21]. Different types of viruses used are: Adenovirus, Adeno-associated virus (AAV), Herpes simplex virus (HSV), Retrovirus (Table 1).

2. NON-VIRAL VECTORS [23]

2.1 Physical

a. Electroporation: It uses high voltage electrical current to facilitate DNA transfer.
b. Micro-injection: Highly efficient technique, and one cell at a time is targeted for DNA transfer.
c. Ballistic transfer of gold micro-particles using gene gun.

Newer method of transfection where DNA was delivered with insertional efficiency and decrease cellular damage are: a) Gene gun, b) Sonoporation, c) Magnetofecation [21].

Table 1. Gene therapy approaches in oral cancer and precancer

Gene or level of action	Vector used/approach	Mechanism of action	Means of administration	Author/year
Mutated or altered P53	Adenovirus ONYX-015	Increases replication in cells with altered p53 (OSCC) by using adenovirus or ONYX-015	Intravenous injection Alone, plus 5-fluoracil or plus IL-2	Nemunaitis et al, 2003
Mutated or altered P53	Adenovirus ONYX-015	Reduction of leukoplakias	Mouthwash: Advexin®	Nemunaitis et al, 2000
Alteration of Rb protein	OAS403	Controls expression of gene E4 and decreases in vivo and in vitro toxicity	Alone or plus Doxil® (chemotherapeutic)	Ryan, 2004
MnSoD gene	Addiction G.T	Suppresses tumour malignity by reducing peroxide flow and therefore cell mitosis	----------	Liu et al, 1997
tKHSV gene	Suicide G.T	Increases apoptosis	----------	Fukui et al, 2001
MDR1, MRP1, DHFR	Suicide G.T	Reduces tumour angiogenesis, increases apoptosis, modifies immune system	----------	Gottesman, 2003
4-1BB gene	Immunotherapy	Activation of T lymphocytes	----------	Cheuck et al, 2004
Anti-ICAM-2	Immunotherapy	Complete regression of oral cavity tumors	----------	Perez et al, 2002
Intratumoral injection of Adv-F/RGD	Immunotherapy	Increases anti-tumour effect by local control of the disease	----------	Denair et al, 2003

Monod: Manganese Superoxide Dismutase; this: Thymidine kinase gene of the Herpes Simplex Virus
MDR1: Multidrug resistant protein 1; MRP1: Multidrug related protein; DHFR: Dihydrofolate-reductase
GT: Gene Therapy; Courtesy: [15]

Table 2. Most frequently used viral vectors

Virus	Advantages	Disadvantages
Retrovirus	- Well known, easily managed - Includes upto 9 kb of exogenous genetic material - Efficient transfer and high levels of expression - Integration in genome ensures permanent expression	- Infects only cells in division; low transduction efficacy - Possible insertion mutagenesis - Low titres - Possible generation of infective viruses
	- Infects dividing and resting cells; high transduction efficacy - Includes upto 7.5 kb of exogenous genetic material - High titres - Does not integrate in the genome, which avoids insertion mutations	- Transitory expression; anti-adenoviral immunity may be less effective, requiring periodic treatments - Lower expression levels - Possible immune and inflammatory reactions - Risk of multiplication
Adeno-Associated Viruses (AAV)	- High transduction efficacy	- Difficult to prepare
Herpesvirus	- Thymidine kinase expression; high efficacy of gene transfer - Capable of inserting large foreign DNA sequences and producing long-term latent diseases - Capable of distributing genes to pleuripotent cells and their differentiated progeny	- Relative toxicity - Gamma-herpes viruses are sometimes related to malignity

Courtesy: [13]

2.2 Chemical [24]

a. Calcium vector: DNA doped calcium phosphate nanoparticles are transferred in encapsulated DNA
b. Lipid vectors: Produced by a combination of plasmid DNA and a solution results in formation of liposome
c. Protein complex: Cell specific DNA delivery systems that utilize unique cell surface receptor on the target cell

Various other chemical delivery systems include a) Oligonucleotide, b) Lipoplexes and Polyplexes, c) Dendrimers, d) Inorganic nanoparticles such as Gold, Silica and Iron oxides are used for gene delivery [21].

3. GENE THERAPY STRATEGIES FOR ORAL CANCER [3]

1. Gene addition therapy (addition of a tumor-suppressor gene)
2. Gene excision therapy (deletion of a defective tumor gene)
3. Antisense RNA (down regulation of the expression of genes that stimulate tumor growth) (Fig. 3)
4. Immunotherapy (enhancement of immune surveillance)
5. Suicide gene therapy (activation of prodrugs with chemotherapeutic effect) (Fig. 4)
6. Introduction of viruses that destroy tumor cells
7. Delivery of drug resistance gene.
8. Introduction of genes to inhibit tumor angiogenesis

3.1 Hurdles in Gene Therapy [1,18,22]

3.1.1 Gene delivery

Successful gene delivery is not easy or predictable, even in single-gene disorders. For example: Genetic basis of cystic fibrosis is not well known, also the presence of mucous in the lungs make it physically difficult to deliver genes to the target lung cells. Delivery of genes for cancer therapy may also be complicated by the disease being present at several sites. Gene therapy trials for X-linked severe combined immunodeficiency (X-SCID) have been more successfully established.

3.1.2 Durability and integration

Main aim is to achieve a long term effect. The therapeutic materials need to remain functional for the intended duration. Two possible ways for this are either use multiple rounds of it or to integrate it so that they remain active for some time. Integrating gene into target cell may achieve long lasting effect, but associated with some undesirable side-effects. Due to this researchers are investigating ways for long-term therapeutic effects without integration. To treat cancer, the aim is to use 'suicide' genes to kill cancerous cells as quickly as possible.

3.1.3 Immune response

When a viral vector is used to deliver gene therapy, the body may recognize them as 'foreign' and mobilize the immune system to attack it. In cancer, triggering such response may be the aim of gene therapy. In other cases, immune responses may reduce the efficacy of it, causing the patient to stop responding after a few applications or by inducing serious side effects, thus make it difficult to give repeat applications of gene therapy.

3.1.4 Safety of vectors

In most of the studies viruses is of choice, presents a variety of potential problems to the patient, e.g., toxicity, immune and inflammatory responses and gene control and targeting issues. Also with the fear that viral vector may recover its ability to cause disease.

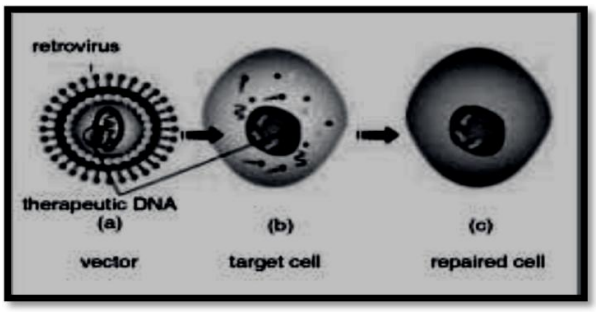


Fig. 2. Viral vector

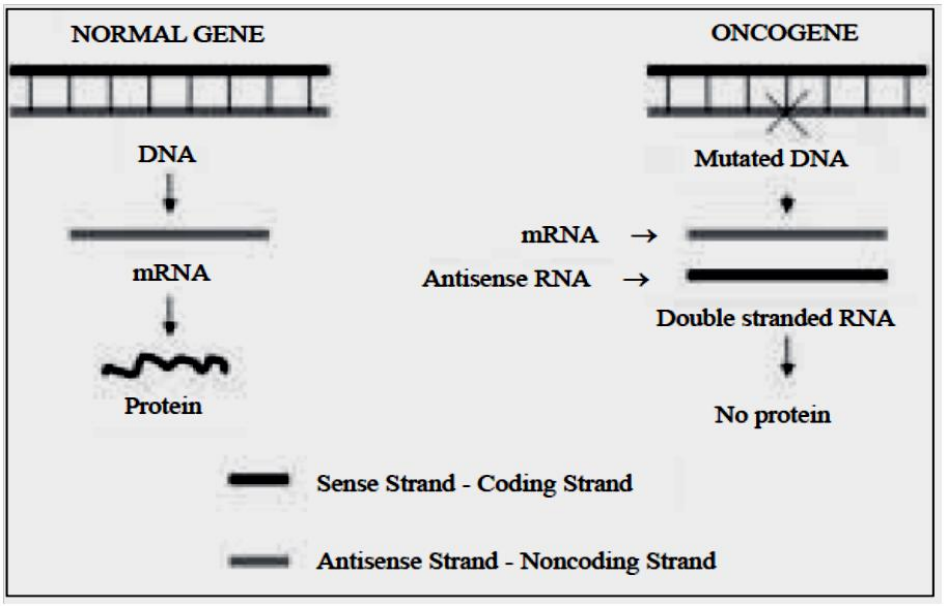


Fig. 3. Antisense RNA therapy

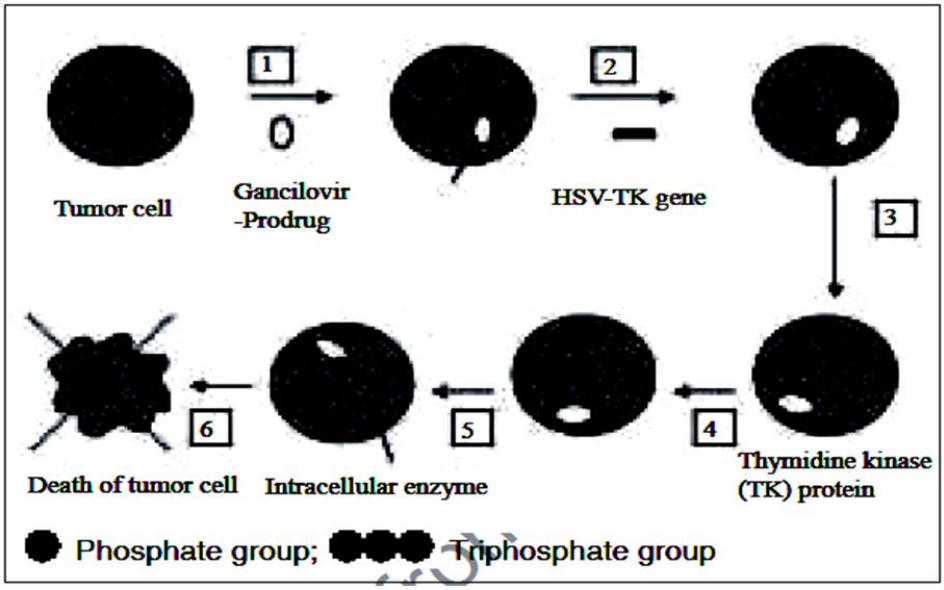


Fig. 4. Suicide gene therapy

3.1.5 Multi-gene disorders

Single-gene mutation disorders are best candidates for gene therapy. Most commonly occurring disorders are caused by the combined effects of variations in many genes such as heart disease, high blood pressure, alzheimer's disease, arthritis and diabetes. Thus difficult to treat effectively using gene therapy.

3.2 Future of Gene Therapy

Gene therapy is still in its infancy, but significant accomplishments have been achieved [25]. Research on gene therapy in oral cancer is increasing in the laboratories and in the clinical settings [2]. This is largely due to the lack of any model in which the virus replicates and spreads until the entire tumor is infected and all cells are destroyed [26]. The ability to transfer genes safely and successfully is yet to achieve. The future of cancer treatment based on the molecular properties of the tumor, utilizing combinations of novel and conventional agents. The revolution in molecular methods has allowed the development of approaches whereby cancer-specific changes can be targeted [25]. The advances and new approaches are still continues in the field and significant improvements are needed for the same [26]. Also as gene therapist, clinician need to appreciate the emerging use of genetics in preventing, diagnosing, and treating dental conditions [27].

4. CONCLUSION

Gene therapy is novel method which should be advocated in treating various diseases including oral cancers. Genetic abnormality associated with various factors which can be treated if further research and study continue in same field. So to conclude, Gene Therapy can be a promising way to treat diseases which has no cure.

CONSENT

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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