GENE THERAPY IN ORAL SQUAMOUS CELL CARCINOMA - A SHORT REVIEW

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Abstract

Oral cancer remains one of the leading causes of death world wide. Various means to destroy tumor cells preferentially have been developed; gene therapy is one among them with less treatment morbidity. Gene therapy involves the transfer of therapeutic or working copy of genes into a specific cell of an individual in order to repair a faulty copy of gene. The alteration can be accomplished by repairing or replacing the damaged DNA by various strategies and vectors. To date genetically altered viruses are commonly used as gene delivery vehicle (vector) which has an advantage of evolutionary selection of host-virus relation. Non viral vectors which include the physical transfection of genes can be accomplished by electrophoration, microinjection, or use of ballistic particles and chemical transfection by forming liposomes.

Keywords : Oral squamous cell carcinoma, gene therapy

Introduction

Oral Squamous Cell Carcinoma (OSCC) is one of the most common malignant tumours seen throughout the world. The morbidity and mortality due to oral squamous cell carcinoma is not yet reduced even after the introduction of traditional treatments like surgery, radiotherapy and chemotherapy. It was documented that OSCC recur in one third of the patients despite of the definitive treatment [1]. Significant proportion of patients remain resistant to the standard therapeutic procedures. Moreover, the treatment will lead not only acute and chronic organ toxicity but also, an increased risk of secondary malignancy.[2] Hence, new treatment modalities are required to improve the overall survival rate and decrease treatment-related morbidity. Gene therapy is an emerging field of biomedicine in which defective gene is replaced and repaired by therapeutic gene. It has the potential to target the cancer cells while the normal tissues are spared. Oral squamous cell carcinoma is an attractive tumor target for gene therapy due to its frequent genetic mutations [3].

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Technique for genetherapy

Gene therapy can be defined as gene transfer for the purpose of treating human disease.[4] This includes the transfer of new genetic material as well as the manipulation of existing genetic material. The objective of gene therapy is to introduce new genetic material into target cells while causing no damage to the surrounding healthy cells and tissues. It has been defined as the "genetic modification of cells of a patient in order to fight a disease". The general strategy is to express a gene product that will result in cancer cell death which include. [3]

(1) Gene addition therapy
(2) Gene excision therapy
(3) Antisense RNA therapy
(4) Immunotherapy
(5) Suicide gene therapy
(6) Gene therapy using oncolytic viruses
(7) Introduction of genes to inhibit tumor angiogenesis.
(8) Delivery of drug resistance gene(s) to normal tissue for protection from chemotherapy.

Delivery systems for gene therapy

Vector is defined as the vehicle that is used to deliver the gene of interest. Vector delivers the therapeutic gene into patients target cell. The target cells become infected with therapeutic gene through vector. Functional proteins are created from the therapeutic gene causing the cell to return to a normal stage.[5] The ideal vector would transfer a precise amount of genetic material into each target cell, thereby allowing the expression of the gene product without causing toxicity[3]. Requirements for vectors are, it should not be identified by the immune system (non immunologic), should be stable and easy to reproduce and have longevity of expression.[6]

The vectors used in gene therapy can be non viral and viral. Non viral vectors include physical transfection of genes which can be accomplished by electroporation, micro injection or use of ballistic particles. Electroporation therapy with intralesional bleo mycin is reported as a technically simple outpatient technique where a high-voltage electric impulse is delivered into a neoplasm by transiently increasing the cell membrane permeability to large molecules like cytotoxic agents, thereby causing localized progressive necrosis. Chemical transfection introduces DNA by calcium phosphate, lipid, or protein complexes. Lipid vectors are generated by a combination of plasmid DNA and a lipid solution that result in the formation of a liposome.[3] Liposomes have no replication risk and are less immunogenic than viruses. The use of cationic liposomes as nonviral vehicles for the delivery of therapeutic molecules is prevalent in the field of gene therapy. Delivery of wild-type (wt) p53 to a radiation-resistant squamous cell carcinoma cell line via ligand targeted liposome complex was also able to modulate the radiation-resistant phenotype of these cells in vitro. These results indicate that this tumor-specific, ligand-liposome delivery system for p53 gene therapy, when used in combination with conventional radiotherapy, may provide a new and more effective means of cancer treatment .[7]

The viruses commonly used in gene therapy are Adenovirus, Adeno-associated virus (AAV), Herpesvirus and Retrovirus. Adenoviral vectors can infect most of cells of the body regardless of their position in the cell cycle. Approximately 90% of human have already formed antibodies against the virus. It has high transduction efficacy and less insertional mutation but has lower level of expression and needs multiple administrations.

Adeno-associated virus (AAV) with low immunogenicity, has no known pathogenicity, targets non-proliferating cells, and may have discrete genome insertion sites. "Suicide" gene therapy has been shown to be feasible in oral cancer cell lines with the use of an AAV vector.[8] Herpes simplex virus (HSV1) has an advantage of reactivation only at site of infection while remaining latent in normal tissues. HSV vectors can accommodate large pieces of foreign DNA and transfer gene rapidly and efficiently. Retroviruses are efficient vectors which can be easily transfected with high level of expression, but it can infect only cells in division and have low transduction efficacy.[9]

Gene addition therapy

It is a technique by which tumour growth is controlled by introducing tumour suppressor genes that inactivate carcinogenic cells. Unlike normal cells, cancer cells demonstrate impaired cell cycle and apoptosis due to mutation. Genetic alterations have been described in oral cancer, including mutations of p53, the retinoblastoma gene (RB1), p16, and p21.[10] P53 is frequently altered during oral

carcinogenesis it was also mutated in precancerous lesions and conditions. For these reasons, p53 is the commonest tumour suppressor gene used in gene therapy, and numerous viral vectors, especially adenoviral vectors, have been developed for its application.[9] A phase III study is currently under the way using adenovirus vector Ad5CMV-p53 by intramucosal injection followed by administration in the form of mouth wash twice a day for 2-5 days. The same protocol is repeated every 28 days and has shown a capacity to inhibit disease progression in precancerous lesions with no toxic effects.[11] Rb (retinoblastoma gene),p27 and mda-7 (melanoma differentiation-associated gene-7) can also be used in gene addition therapy for their apoptotic and anti tumour effects.

Gene excision therapy

Gene excision therapy is a technique which involves removal of defective oncogenes, thereby inhibiting the growth of tumour cells. Okadaic acid a highly toxic polyether is used to inhibit tumour growth by reducing expression of Egr-1 (early growth response factor 1). Inhibition of Egr-1 may be 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.[12]

Anti sense RNA therapy

Antisense RNA prevent the activity of oncogenes like myc, fos and ras and also inhibit growth of viruses like HSV-1, HPV (Human Papillomavirus) and HTLV-1 (Human T-lymphotropic virus).[13] Antisense RNA can check tumour growth usually by inhibiting RNA that is complementary to the strand of DNA expressing the gene. Major limitation of conventional antisense RNA is inability to introduce sufficient quantities of antisense molecules to down regulate the target gene and inhibit tumor growth.[14] Xi and Grandis has developed an antisense approach which interfere with the autocrine pathway of oral cancer involving the epidermal growth factor receptor (EGFR) its ligand and transforming growth factor alpha (TGFa).[3]

Immunotherapy

The goal of immunotherapy in oral cancer involves either increasing the immunogenic potential of tumor cells or augmenting the patient's immune response to a tumor. Patients with oral cancer show deficient function of several types of immune cells which include natural killer cells, T-lymphocytes, and cytokines.[10]

The combined use of mIL-2 (murine interleukin 2)and mIL-12(murine interleukin-12) gene therapy resulted in significant reduction in the tumour due to increased activation of cytolytic T lymphocyte and natural killer cells.[15] Radiosensitivity to γ radiation and chemosensitivity to 5-fluoracil (5-FU) in OSCC can be enhanced after suppression of NF-kB activity, which activates the antiapoptotic proteins TNF, TRAF-1, TRAF-2 and cIAP-1. The inhibition of NF-kB can decrease expression of proinflammatory cytokines, e.g., IL-1α, IL-6 and IL-8, and of enzymes that degrade matrix metalloproteinase-9 (MMP-9). The progression and metastasis of OSCC can be achieved by inhibiting NF-kB activity which may be a useful coadjuvant treatment in oral cancer therapy.[16] Systemic administration of Anti-ICAM-2 induced the complete regression of OSCC lesions. ICAM-2 is a glycosylated protein with surface adhesion that is expressed in endothelial cells and activates lymphocytes. However, the regression of tumour is dependent on the immune system function and the induction of specific tumour toxins by the action of CD8 lymphocytes.[17]

Suicide gene therapy

Suicide gene therapy involves the expression of enzymes that can transform non-toxic producing drug into active cytotoxic substances. The thymidine kinase gene of Herpes Simplex Virus (HSV) transforms ganciclovir into ganciclovir phosphate there by

killing cancer cells.[18] The suicide gene therapy using the herpes simplex virus thymidine kinase (HSVtk) gene via adenovirus vector followed by ganciclovir (GCV) administration induced remarkable cytotoxicity with a bystander effect in human oral squamous cell carcinoma thus suggesting an effective treatment strategy for OSCC.[19] The disadvantages of suicide gene therapy is the poor distribution of the vector within the tumour and poor transfection efficacy.

Gene therapy using Oncolytic viruses

It is a novel approach to gene therapy that involves a vector that selectively replicates within and lyses tumor cells. The virus should be genetically modified to attenuate its toxicity in normal tissue while maintaining its oncolytic activity against malignant tumours, without compromising the safety and anti-tumour efficacy.[20] The anti-tumoural efficacy of ONYX-015 was less, when it was used alone in patients with recurrent squamous cell carcinoma.[21] The combination of intra-tumoral ONYX-015 injection with cisplatin and 5-fluorouracil in patients with recurrent squamous cell carcinoma showed better results.

Patients treated with Advexin® mouthwash (Introgen Therapeutics, Inc (INGN), NY), a commercial product which also administers p53 by means of an adenovirus, showed a marked decrease in the number and aggressiveness of precancerous cells in epithelial dysplasia.[22] Intravenous injection of oncolytic adenovirus OAS403 showed cytotoxicity in tumour cells especially where Rb protein and the regulation of telomerase expression is altered.[23]

Introduction of genes to inhibit tumor angiogenesis

Angiogenesis is a prerequisite for the development of solid tumors. Tumour cell invasion on newly formed blood vessels induce tumour progression. Scientists are developing vaccines against receptor 2 of the VEGF factor (Vascular Endothelial Growth Factor) also known as FLK-1, there by inhibiting angiogenesis, tumour growth and metastasis. The vaccine against FLK-1 is effective, stimulating T lymphocytes that inactivate this receptor. This vaccine will be useful in the treatment of tongue metastasis of OSCC, with an increased immune response.[24]

Delivery of drug resistance gene(s) to normal tissue for protection from chemotherapy

Drug resistance genes will protect normal tissues while leaving malignant cells vulnerable to destruction. The most widely studied drug resistance gene is human Multidrug Resistance-1 (MDR-1) gene. Several other drug resistance genes are currently under study which include the bacterial nitroreductase gene (which protects against drugs such as thiotepa) and dihydrofolate reductase mutants which protect against methotrexate /trimetrexate.[25,26]

Conclusion

Gene therapy will be an attractive tool in the treatment of oral squamous cell carcinoma and precancer because it targets cancer cells while sparing normal tissues. The clinical application of gene therapy for treatment of oral cancer will require optimization of gene delivery. Phase II clinical studies by various investigators have showed promising results when gene therapy combined with chemotherapy, radiation therapy or surgery. Such augmentative approaches, will reduce morbidity of the treatment and help maintain quality of life in patients with oral squamous cell carcinoma. In future instead of supplement therapy it may even become the one of the conventional cancer therapeutics. Phase III clinical trials is presently under way, further investigation is warranted to establish safe and effective approaches that utilize gene therapy

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Erratum

1. George A, Sreenivasan BS, Sunil S, et al. Potentially Malignant Disorders of Oral Cavity. Vol 2, No 1, Jan- Jun 2011; 95-100 : The error in the manuscript has been rectified in the online version. (www.ompj.org).
2. Geetha Varghese, Pradeesh Satyan; Hypohidrotic ectodermal dysplasia- A case study, Vol2 No1, Jan- Jun 2011;123-6: The error in the manuscript has been rectified in the online version. (www.ompj.org).