Gene therapy in the management of oral cancer: Review of the literature

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Abstract

Gene therapy essentially consists of introducing specific genetic material into target cells without producing toxic effects on surrounding tissue. Advances over recent decades in the surgical, radiotherapeutic and chemotherapeutic treatment of oral cancer patients have not produced a significant improvement in patient survival. Increasing interest is being shown in developing novel therapies to reverse oral epithelial dysplastic lesions. This review provides an update on transfer techniques, therapeutic strategies, and the clinical applications and limitations of gene therapy in the management of oral cancer and precancer. We highlight the combination of gene therapy with chemotherapy (e.g., 5-Fluoracil) and immunotherapy, given the promising results obtained in the use of adenovirus to act at altered gene level (e.g., p53). Other techniques such as suicide gene therapy, use of oncolytic viruses or the use of antisense RNA have shown positive although very preliminary results. Therefore, further research into these promising gene therapy techniques is required to assess their true efficacy and safety in the management of these lesions.

Key words: *Gene therapy, oral cancer, viral vectors.*

Introduction

Oral cancer (OSCC, for Oral Squamous Cell Carcinoma) is a genetic disease in which the genes that control cell growth and apoptosis are mutated, allowing cells to acquire the ability to invade and metastasize. Despite research efforts and new therapies, five-year survival has not improved over the past 4-5 decades. Patients with recurrent oral cancer that is refractory to chemotherapy or radiotherapy have a life expectancy of only months and the response rate to second- and third-line treatments is only 15% (1).

This situation could drastically change over the next few years, thanks to the revolution in our knowledge of the disease brought about by molecular studies, which have already allowed us to differentiate between premalignant and malignant conditions (1). The introduction of new genes and the activation or inactivation of others may inhibit or suppress tumour growth (2). Gene therapy can potentially attack cancerous cells while respecting normal tissue. It may be useful to manage disease recurrence and as a coadjuvant therapy, e.g., in resected tumour margins. Clinical application of this technique in the treatment of oral cancer and precancer requires optimization of viral vectors and improvement of transfection effectiveness.

The aim of this review is to analyze the different modalities of gene therapy currently used to manage precancerous and cancerous lesions of the oral cavity. They include addiction gene therapy, suicide gene therapy, immunotherapy, oncolytic virus therapy, inhibition of tumour angiogenesis, gene deletion therapy and antisense RNA.

Concept of gene therapy

The objective of gene therapy is to introduce new genetic material into target (cancerous) cells while causing no damage to surrounding healthy cells and tissue. It has been defined as the "genetic modification of cells of a patient in order to fight a disease"(3). Gene therapy includes both the transfer of new genetic material and the manipulation of existing genetic material. At the present time, the most widely used gene therapy procedure follows these steps: (i) identification, isolation and amplification of the gene to be used in the treatment; (ii) extraction and in vitro culture of tissue cells from the patient to be treated; (iii) transfer of the therapeutic gene into these cells via a vector (Table 1), using a gene that contains a promoting sequence to enable its expression and a marker to identify cells into which it is incorporated; and (iv) transfer into the patient of selected gene-containing cells. The theory is that when the gene exerts its normal physiological functions, the disease will be eliminated.

Gene therapy approaches to oral cancer and precancer

OSCC is a good candidate for gene therapy because primary and recurrent lesions are readily accessible for injection or application of the agent (3). Current gene therapy approaches include:

- *Addiction gene therapy:*

The aim of this approach is to regulate tumour growth by introducing tumour suppressor genes that inactivate carcinogenic cells. 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 (4). Moreover, the percentage of epithelial cells expressing mutated p53 is usually higher with greater severity of the epithelial disorder. For these reasons, one of the tumour suppressor genes most commonly used in gene therapy is the p53 gene, and numerous viral vectors, especially adenoviral vectors, have been developed for its application. Table 1

Table 1. Most frequent vectors in gene therapy (Taken from Smith, 1995) (30).

VIRUS	ADVANTAGES	DISADVANTAGES
RETROVIRUS	-Well known, easily managed - Includes up to 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
ADENOVIRUS	- Infects dividing and resting cells; high transduction efficacy - Includes up to 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 pluripotent cells and their differentiated progeny	- Relative toxicity -Gamma-herpes viruses are sometimes related to malignity

summarizes the most used vectors in gene therapy, with their advantages and disadvantages.

Some authors analysed the efficacy of gene p53 to inhibit tumour growth as a function of the use or not of adenovirus vectors with replicative capacity. Results showed that efficacy was higher when the replicating vectors were used, although the reasons for this improvement are not fully understood (5). A phase III study is currently under way on adenovirus vector Ad5CMV-p53. This is applied by intramucosal injection followed two hours later by a mouthwash. From the next day, it is administered as a mouthwash twice a day for 2-5 days. This treatment is repeated every 28 days and has shown a capacity to inhibit disease progression in precancerous lesions with no toxic effects (6).

Other tumour suppressors introduced into tumour cells by gene transfer are Rb (retinoblastoma gene) and mda-7 (melanoma differentiation-associated gene-7). Their effects on apoptosis and tumour growth appear to be similar to those of interleukin-24 (Il-24), a Th1 cytokine that triggers an anti-tumour and pro-apoptotic response in the immune system (7).

Gene transfer of 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 appearance of tongue cancer. According to these results, the therapeutic use of p27 gene may in the future prove useful for the treatment of OSCC (8).

Recent mouse studies found that the intratumoural injection of Allovectin-7® (Vical Inc, San Diego, USA), suppressed the growth of head and neck tumours. Allovectin-7 is the result of gene transfer, i.e., co-expression of the human gene of the HLA-B7 leukocyte antigen with beta2-microglobulin gene. This treatment appears to be relatively well tolerated, with only mild or moderate reactions around the injection site that are reduced after the second injection. However, further studies are required since only preclinical trials in mice have been performed to date (9).

Gene therapy using oncolytic viruses

One of the most promising gene therapy approaches is the use of viruses that replicate only tumour cells, designated oncolytic viruses. This procedure emerged from the discovery of adenoviruses lacking E1B, which did not grow in normal cells but grew in cells without p53, one of the most common characteristics of tumour cells. Adenovirus ONYX-015, which presents deletion of the E1B region, has been used to control OSCC lesions (10).

The release of an oncolytic herpes virus in a primary tumour after its surgical excision appears to significantly reduce the tumour and regional metastasis (11). Therefore, the virus must be genetically modified to attenuate its toxicity in normal tissue while maintaining its oncolytic activity against malignant tumours, with the aim of reinforcing safety without compromising the anti-tumour efficacy of the virus (7). Replication of ONYX-015 adenovirus is minimal in cells with normal p53 function but reaches high levels in cells with p53 alterations or mutations. Intravenous injection of this vector produces important tumour regression and improves survival in presence of metastasis. These oncolytic viruses have shown enhanced activity when combined with chemotherapy. Thus, the use of ONYX-015 alone had an efficacy of 20%, which was increased by up to 6% in combination with 5-fluoracil (5FU) (12). Presence of cytokines IL-6, TNF-α, IL-10 and IFN-γ is observed at 24 hrs after ONYX-015 administration. IFN-γ levels increase after four days, while TNF-α levels increase after six hrs, thereby contributing towards the appearance of an immune response to the tumour (13).

Patients with precancerous lesions were treated with Advexin® mouthwash (Introgen Therapeutics, Inc (INGN), NY), which also administers p53 by means of an adenovirus. Tissue analyses before and after treatment showed a marked decrease in the number and aggressiveness of precancerous cells. This treatment, currently in a phase II trial, also appears to be very well tolerated (14). Rudin et al tested three different dose and duration regimens 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 (15).

Other researchers have reported on the anti-tumour efficacy of the intravenous injection of oncolytic adenovirus OAS403. Cytotoxicity appeared in tumour cells at 7-10 days after exposure to this adenovirus. This adenovirus is especially active in tumours with alterations in Rb protein and in the regulation of telomerase expression. This adenovirus has proven to be selective, innocuous and efficacious in both in vivo and in vitro trials, and its systemic administration has been shown to be highly effective against tumours in mice (16). Again, the combination of OAS403 with conventional chemotherapeutic agent, e.g., liposomal doxorubicin, increased the anti-tumour efficacy in preclinical studies, and Phase III trials are now under way. Table 2 summarises current gene therapies that use oncolytic viruses.

- *Suicide gene therapy:*

In suicide gene therapy, genes are introduced that stimulate the generation of products that are toxic for the cells. When suicide genes are used in retroviral vectors, protection measures must be taken against the appearance of oncogene-activating mutations (3). Suicide genes permit the expression of enzymes that can transform non-toxic drugs into cytotoxic substances. Thus, the thymidine kinase gene of Herpes Simplex Virus (HSV) transforms ganciclovir into ganciclovir phosphate (2).

Suicide gene therapy can also use adenoviruses, although, as mentioned above, some characteristics of these vectors limit their efficacy and safety (Table 1) (17). Nevertheless, 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 (18).

One of the main drawbacks of suicide gene therapy is the poor distribution of the vector within the tumour. Although a relatively low anti-tumour response has been observed in clinical trials because of poor transfection efficacy, a high percentage of transfected cells do not appear in vivo due to their ability to induce tumour death (3).

Considerable knowledge is now available on genes that contribute towards resistance against chemotherapy, including MDR1 (Multidrug resistance protein 1), MRP1 (Multidrug Related Protein) and DHFR (Dihyfolato-reductase) (19). In these cases, gene therapy has three main objectives: action at tumour angiogenesis level; protection of normal tissue, especially medulla, from toxic effects of chemotherapy; and enhancement of the immune system (2). (See Table 2)

- *Introduction of genes to inhibit tumour angiogenesis*

Research is in progress on the use of microencapsulated cells in antiogenic cancer therapy. The technique consists of the release of therapeutic proteins to encapsulate recombinant cells. Microcapsules are designed to be permeable to recombinant products and nutrients but not to host immune mediators, due to their large size. These cells are capable of secreting angiostatin, an important angiogenic inhibitor. Angiostatin receptors present ATP synthase on the surface of human endothelial cells, as in the case of αvβ3-integrin and vitronectin. This enables angiostatin to be localized in the tumour instead of organs near the implantation site of the capsule. However, this technique is not adequate for long-term removal of the tumour, especially when it is in an advanced stage. Moreover, it is a prolonged therapy that requires repeated doses and is associated with a high degree of toxicity (20).

Investigators are also developing vaccines against receptor 2 of the VEGF factor (Vascular Endothelial Growth Factor), also known as FLK-1, with the resulting inhibition of angiogenesis, tumour growth and metastasis. Studies have demonstrated that the vaccine against FLK-1 is effective, stimulating T lymphocytes that inactivate this receptor and, therefore, vascularisation of the tumour. This vaccine also appears to be useful in the treatment of tongue metastasis of OSCC, with an increased immune response observed at 10 months of the inoculation (21).

- *Immunotherapy*

The aim of immunotherapy is to increase the patient's immune response to the tumour. Patients with OSCC present altered function of immune cells, including NK cells, T lymphocytes and numerous cytokines. Thus, animal studies have shown that IL-2 administration activates T lymphocytes and NK cells and that these in turn activate tumour necrosis factor α (TNF-α), triggered by the strong tumour inhibition effect (3).

Mechanisms to increase the sensitivity of the tumour to normal therapeutic processes are under investigation. 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 also increases the expression of proinflammatory cytokines, e.g., IL-1α, IL-6 and IL-8, and of enzymes that degrade matrix metalloproteinase-9 (MMP-9). 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 (22).

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) (23). The intratumoral injection of Adv-F/RGD showed a high anti-tumour effect due to increased mononuclear cell infiltration and major necrotic changes. It also achieved local control of the disease, an essential objective since most deaths result from metastasis. The development of a new gene therapy for the local treatment of oral cancer would be extremely valuable (24).

Ongoing research has reported that adenoviruses OW34, E1B55KD and HSV-TK were more effective in combination with ganciclovir than when used alone (3).

Another therapeutic approach may be to use the monoclonal antibody Anti-ICAM-2 alongside the intratumoural gene transfer of interleukin-12. ICAM-2 is a glycosylated protein with surface adhesion that is expressed in endothelial cells and activates lymphocytes. Recent studies found that systemic administration of Anti-ICAM-2 induced the complete regression of OSCC lesions. However, the tumour regression is dependent on the immune system function and the induction of specific tumour toxins by the action of CD8 lymphocytes (25).

Gene transfer of IL-12 using plasmid pNGVL3-mIL12 is also being investigated. An FDA-approved clinical trial is under way but it is too early to predict results (26). (See Table 2)

- *Excision gene therapy*

The aim of this therapy is to remove defective oncogenes, thereby inhibiting the growth of tumour cells. Thus, the efficacy of using akadaic acid to suppress Egr-1 (early growth response factor 1) protein expression is being studied in the OSCC setting. Okadaic acid 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, since it is related to cell proliferation and division. Inhibition of Egr-1 may represent a good therapeutic approach, since genes that

Table 2. 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 tumours		Pérez et al, 2002
Intratumoural injection of Adv-F/RGD	Immunotherapy	Increases anti-tumour effect by local control of the disease	----------	Dehari et al, 2003

MnSOD: Manganese Superoxide Dismutase
tKHSV: Thymidine kinase gene of the Herpes Simplex Virus
MDR1: Multidrug resistant protein 1
MRP1: Multidrug related protein
DHFR: Dihydrofolate-reductase
GT: Gene Therapy

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. Some authors demonstrated that inhibition of protein kinase C reduces the expression of this gene, triggering higher sensitivity of the tumour to radiotherapy (27).

- *Antisense RNA*

Gene expression can generally be inhibited by RNA, which is complementary to the DNA. 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). Conventional use of this technique is limited by the difficulty of introducing a sufficient quantity of antisense molecules to inhibit tumour growth. Powerful promoters are currently being developed to overcome this drawback. Thus, preclinical studies using antisense sequences under the control of six small RNA promoters demonstrated a powerful anti-tumour effect with minimum toxicity (28). A phase 1 trial is under way in patients with advanced oral cancer to evaluate the safety and biological effects of administering liposome-mediated intratumoral EGFR (Epidermal Growth Factor Receptor) by means of antisense gene therapy. Results have been positive, showing a low toxicity and high efficacy (29).

In general, positive outcomes have been reported for gene therapy (30). Only three adverse effects have been associated with gene therapy in oral cancer, including one death. It should also be borne in mind that patients undergoing this type of treatment 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 (3).

The most frequently observed adverse reactions are severe inflammatory processes and coagulopathies, generally in relation to the viral vectors employed. In order to predict these effects, the FDA and NIH have developed a program: The Genetic Modification Clinical Research Information System (GeMCRIS) www.gemcris.od.nih.gov/, an accessible web-based system that compiles and analyses all data related to gene therapy (2).

Conclusions

As shown in this review, research on gene therapy in oral cancer is increasing in the laboratory and in the clinical settings. In the medium- and long-term, it may contribute a definitive treatment for oral cancer and precancer 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 appear to be the most promising approaches to the management of oral cancer and precancer.

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