

Gene Therapy in Head and Neck Cancer

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ABSTRACT

Advancements in the field of molecular biology have stimulated the interest of the scientists and clinicians worldwide who believe that genetic manipulation can be a potential cure for cancer. Gene therapy is one such therapy that is significantly applied in human cancer which involves transferring genetic material into a host cell via a viral or non-viral vector, immunotherapy or by manipulation of the tumor microenvironment to reduce angiogenesis. It is not only being considered as a therapeutic strategy to treat cancer but also as a medium to counter the post radiation side-effects. More recently it has been used as a means to prevent the progression of potentially malignant diseases into cancer thereby proving to be a potential candidate for improving survival rates in cancer patients. This review aims to provide insight into the field of cancer gene therapy as applicable to oral squamous cell carcinoma and oral premalignancies that may have far reaching effects on the way a cancer patient is managed.

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INTRODUCTION

Head and neck cancer is the seventh most common cancer worldwide with more than 635000 new cases of this cancer being diagnosed globally with 350000 related deaths every year. (Ferlay, Shin et al 2010)^[1,2]. Oral squamous cell carcinoma (OSCC) represents 95% of all forms of head and neck cancer, and during the past decade its incidence has increased by 50%^[3]. Despite the advances in the currently available therapeutic strategies, which include surgery either alone or in combination with radiotherapy and chemotherapy, the five year survival rate is less than 50%. In addition, a high percentage of patients have a poor response to therapy and high recurrence rates^[3]. Despite the plethora of treatment options available, most lesions are not completely cured and have a tendency to recur. There are limited treatment options for recurrent or refractory oral cancers. The high morbidity and mortality associated with head and neck cancer led to the introduction of cancer gene therapy which refers to the introduction of new genetic material to human cells for a therapeutic purpose. In the early years, gene therapy was focused on monogenic diseases such as severe combined immunodeficiency, hemophilia and certain enzyme deficiencies which could be corrected by replacing the "missing gene" but more recently clinical trials for gene therapy have been directed towards cancer (64.3%) with a total of 1,223 trials being carried out globally^[4,5].

The idea of gene therapy was introduced by Joshua Lederberg in 1963; however, research on human genetics gained momentum only much later in the 1980s^[6]. The first FDA-approved successful gene therapy treatment and clinical study in the United States occurred almost 25 years ago, in 1990 for a patient with X-linked severe combined immunodeficiency disorder and ever since gene therapy showed promising results in various diseases like chronic lymphocytic leukemia, acute lymphocytic leukemia and brain tumors^[7,8]. Most of the approved European and United

States gene therapy protocols are for cancer (~66%), in contrast to monogenetic diseases (~11%) and cardiovascular diseases (~8%) and the focus of cancer gene therapy has been with respect to melanoma, prostate, ovarian cancers and leukemia^[9]. In India, the first gene therapy research was performed by Rita Mulherkar's group (1998) from ACTREC related to the treatment of head and neck cancer using viral vectors. This group focused on preclinical studies using xenograft mouse models to test the combined efficacy of herpes simplex virus-thymidine kinase gene and Ganciclovir treatment. Rupesh Dash, began his work related to cancer gene therapy in 2012 and is currently working on systemic and targeted gene therapy for cancer, particularly the anti-apoptotic Bcl-2 family members towards an effective therapy for oral squamous carcinoma^[10].

In the oral region the focus of gene therapy was mainly on primary treatment of oral squamous cell carcinoma but currently the salivary glands are also being regarded as a promising target thus enabling the repair of gland damage which usually follows therapeutic radiation for head and neck cancers^[11]. Also the successful clinical trials of gene therapy in oral cancer patients have further made scientists believe that gene therapy can be an important tool in combating potentially malignant diseases thereby preventing malignant transformation. The aim of this review is to shed light on gene therapy as a treatment modality for cancer, as a means to counter the side effects of radiotherapy and a tool to prevent malignant transformation of potentially malignant disorders.

GENE TRANSFER CONCEPT AND DELIVERY SYSTEM

The term gene transfer refers to the delivery of a gene, a cDNA, a small RNA, i.e., any type of oligonucleotide that might have some therapeutic benefit, to a pre-determined target cell^[11]. The objective of gene therapy is to introduce new genetic material into target cells while causing no harm to the surrounding normal tissue. There are two main approaches: In vivo gene transfer where genes are delivered directly to target cells in the body and Ex vivo gene transfer wherein target cells are genetically modified outside the body and then reimplanted^[12]. The transfer of genetic material into the target cells is brought about mainly by viral and the non-viral vectors. The viral vectors include a host of viruses such as retrovirus, adenovirus, adeno-associated virus, herpes virus and lentivirus. The viral vectors have been accounted for the majority of the clinical trials and considered promising for treating cancer especially when combined with chemotherapy^[9]. In gene therapy, modified versions of adeno virus and adeno-associated viral vectors have been designed. They replicate exclusively in tumor cells and selectively target specific cellular receptors. They are more potent in infecting cells as compared to their wild-type counterparts^[13]. The non-viral vectors include the plasmid DNA and lipofection among others^[6]. Non-viral vectors are much safer and can be constructed and modified by simple methods, and exhibit high gene encapsulation ability as compared to their viral counterparts but not as efficient as them^[7]. In HLAB7negative melanoma patients, antitumor immunity is induced by injecting cationic liposomes containing HLAB7 and β -2 microglobulin encoding genes^[14].

GENETIC BASIS OF CANCER

Cancer is a multistep process that develops through accumulation

of genetic and epigenetic alterations (Ha *et al* 2009). These genetic alterations include loss, gain or translocation of chromosomal segments, activation of proto-oncogenes, or inactivation of tumor suppressor genes. The key element tumor suppressor gene p53 gene is mutated in approximately 60-80 % of HNSCC and the p16 gene is altered in 50-80 % of cases by homozygous deletion, promoter methylation or point mutation^[15]. The p53 protein blocks cell division at the G1 to S boundary, stimulates DNA repair after DNA damage, and also induces apoptosis^[16]. Wild-type p53 gene inhibits carcinogenesis by maintaining genomic integrity. Therefore, p53 gene is also called as "housekeeping gene"^[17]. Wild-type p53 has a very short half-life (four to five minutes), whereas mutant forms of protein are more stable, with a six hour half-life^[16]. p53 mutation arises either as a point mutation, resulting in a structurally altered protein thereby inactivating its tumor suppressor activity, or by deletion, which leads to a reduction or loss of p53 expression and hence the protein function. The function of normal p53 protein is to stagnate cells with DNA damage at stage G1 for DNA repair is thus disturbed^[16,17]. The proto-oncogenes involved in the head and neck carcinogenesis are Her2/neu and cyclin D1. It is no myth that immune cells can kill cancer cells but cancer cells avoid this immune surveillance by suppressing the body's immune system resulting in immunosuppression in HNSCC patients. Gene therapy can be used to stimulate immunity of cancer patients with the harvested autologous tumor cells which have been transduced in vitro with genes encoding cytokines or highly antigenic protein genes. Immunotherapy via gene therapy can be directly instituted by the addition of a tumor antigen or other stimulatory gene to the patient's bone marrow. These cells are thus primed to cause an immune reaction to the cancer cells resulting in the inhibition of tumor growth^[18]. The tumor microenvironment plays a crucial role in tumor progression and angiogenesis is vital for the growth of the lesions. Angiogenesis which is considered as the hallmark of cancer is controlled by the interplay of angiogenic and anti-angiogenesis factors. Attempts have been made to inhibit tumor growth and metastasis by blocking the process of angiogenesis with gene therapy. Recently oncolytic viruses have been armed with anti-angiogenic factors such as endostatin/angiostatin^[19]. Thus the gene therapy treatment strategies may include replacement of defective tumor suppressor genes, inactivation of oncogenes, stimulating the immune response and targeting tumor vasculature to avoid angiogenesis.

APPLICATIONS OF GENE THERAPY IN HNSCC

AS AN ANTI-CANCER THERAPEUTIC AGENT:

Oncolytic virotherapy

Oncolytic viruses are those that are able to replicate specifically in and destroy tumor cells and this property is either inherent or genetically engineered. Adenoviruses have been the most promising gene delivery system which is approved to be used in conjunction with chemotherapy in HNSCC. One such oncolytic adenovirus, "ONYX-015" has been engineered to lack the viral E1B protein and the absence of this protein renders the virus incapable of replicating in healthy cells with normal p53 pathway. It grows in cells without p53 gene which is a hallmark of the tumor cells^[20]. A phase two clinical trial on 11 patients revealed selective "ONYX-015" presence and/or replication in the tumor tissue of 7 of 11 patients but not in immediately adjacent normal tissue. Significant tumor regression (>50%) occurred in 21% of evaluated patients,

with no toxicity to the injected normal peritumoral tissues. Thus the p53 mutant tumors were significantly more likely to undergo “ONYX-015”-induced necrosis than were p53 wild-type tumors^[21]. Its combination with chemotherapy is a promising approach in head and neck cancer^[22]. Better patient response was noted in recurrent head and neck cancer patients when a combination therapy of “ONYX-015” with 5-fluorouracil and cisplatin was instituted. In spite of the fact that the replication of viruses almost always takes place in healthy and rapidly growing cells and that a cytotoxic drug tends to decrease the effects of a virus resulting in the reduced effectiveness of such a combination, there have been positive results in the clinical trials carried out. The effectiveness of such combinations can be attributed to the fact that these might have been demonstrated only in mutant viruses lacking some essential function. The drug might stimulate the tumor cells to express that function and complement the virus’s deficiency. Another popular oncolytic adenovirus termed Gendicine for treating head and neck cancer in China emerged in 2003 (Pearson et al, 2004) but there was not much evidence regarding its continued use as a treatment modality in the recent past^[11].

Addition gene therapy

This approach introduces tumor suppressor genes into the tumor thereby inactivating the cancer cells. The most notable tumor suppressor gene involved in this therapy is the p53 gene. A phase III study is currently in progress on adenovirus vector Ad5CMV-p53^[23]. In a study conducted by Li et al (2014)^[24], wherein a combination of recombinant adenoviral p53 gene therapy and intra-arterial delivery of chemotherapeutic agents for OSCC patients was assessed, it showed a greater treatment response as compared with either treatment alone. The other tumor suppressor genes introduced are p27, Rb, MDA7.

Suicide gene therapy

Suicide gene therapy, also known as gene directed enzyme pro-drug therapy involves transformation of a non-toxic compound into a lethal drug by introduction into tumor cells of a viral or bacterial gene. 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 oral squamous cell carcinoma^[25]. When anti-viral prodrug ganciclovir (GCV) is applied to HSV-tk expressing cells, GCV is converted into monophosphate form by HSV-tk, and eventually into cytotoxic triphosphate derivatives by cellular kinases. Actively dividing cells will be killed as they integrate the nucleotide derivatives into their genome^[26]. Cellular changes like chromatin condensation, cell shrinkage and blebbing of cell membrane was reported when GCV was introduced in the oral squamous cell carcinoma cell lines.

Immunotherapy

Immunotherapy is the principle of boosting an individual’s immune system to destroy the tumor cells. Gene therapy has been used to make recombinant cancer vaccines. The immunosuppression in HNSCC patients is countered by administration of certain cytokines like IL2 via gene therapy that triggers the production of TNF alpha which is a tumor growth inhibitor^[18]. Another example is the gene CYP2B1 which activates cyclophosphamide to a toxic form and it has shown to kill oral cancer cells efficiently in culture and reduce the growth of tumors in animals but no human clinical trial has demonstrated its efficacy^[27].

Antisense RNA therapy

The antisense RNA inhibits the RNA, which is complementary to the DNA and hence the expression of gene associated with tumor growth can be controlled by them. Inhibition of oncogenes such as myc, fos and ras and viruses like HSV-1, HPV, HTLV-1 can prevent the tumor growth^[25].

AS POST TREATMENT MODALITY FOR BETTER QUALITY OF LIFE

Despite the fact that gene therapy as a treatment modality for cancer is the latest and a promising tool for the management of cancer patients, and the extensive research and the continuing clinical trials rendering it safe and efficacious, it is still not viable for the common man. The standard treatment modalities such as surgery, radiotherapy and chemotherapy continue to remain the backbone of oral cancer therapy. Irradiation damage to salivary glands is a common consequence of radiotherapy for HNSCC patients which leads to a loss of acinar cells thereby causing glandular hypo-function. This further leads to xerostomia and adverse oral cavity changes which results in the diminished quality of life. Conventional therapy for salivary hypo-function is only effective if the patient has sufficient functional acinar tissue. However, most of the post irradiation patients have insufficient acinar cell mass which has led to the consideration of use of gene therapy for salivary hypo-function post radiotherapy in HNSCC patients^[11].

Salivary gland is considered as an attractive target for gene based therapy firstly because of its easy access and the ductal orifices of the glands open into the mouth. Secondly, the arrangement of all the parenchymal cells in a monolayer lining the luminal system and the apical membrane of all acinar and ductal cells facilitates direct contact from the oral cavity. Thirdly, they comprise of highly organized protein producing cells. Also the concern of vector spread beyond the glandular tissue is minimized since the glands are well-encapsulated^[28,29].

The radiotherapy damages the fluid and the salt secreting acinar cells whereas the salt absorbing water impermeable ductal cells remain resistant to this radiation damage. It was theorised that the ductal cells should be enabled with water channel proteins for the permeability of water. This resulted in the reengineering of ductal cells by transferring the c DNA of a gene called human Aquaporin-1 via an adenoviral vector resulting in a recombinant adenovirus encoding human aquaporin-1 termed AdhAQP1. The other commonly used vector is the retrovirus. The human aquaporin gene encodes a plasma membrane protein that enables transmembrane water movement in response to osmotic gradient. Post administration of AdhAQP1, the water impermeable ductal cells became water permeable and thus more saliva was secreted into the mouth. In the first human clinical trial, in 11 head and neck cancer survivors, aquaporin1 gene therapy was instituted and it was found that six participants had increased levels of saliva secretion and there were reduced subjective complaints in the other five subjects^[11].

IN ORAL POTENTIALLY MALIGNANT DISORDERS

It is a well-established concept that cancer development in the oral mucosa is a two-step process, i.e., the initial presence of a precursor (pre-malignant, pre-cancerous) lesion subsequently developing into cancer. This concept is supported by various studies that have shown that between 16 and 62% of oral carcinomas are associated with leukoplakic lesions when diagnosed and an Indian study

showed that about 80% of oral cancers were preceded by oral precancerous lesions or conditions^[30]. The lesions in the oral cavity and oropharynx that clearly represent precancerous fields are erythroplakia, leukoplakia and, to some extent, lichen planus^[31]. Literature data indicate that patients with oral submucous fibrosis are at least 19 times more likely to develop OSCC than healthy people^[32]. These patients need more frequent clinical monitoring to detect incipient lesions and may be ideal candidates for chemoprevention to reduce the risk of new tumor onset^[31].

The premalignant oral lesions are particularly suitable targets for gene therapy because of various reasons - firstly, the superficial nature of such precancerous lesions allows easy access for gene delivery and clinical evaluation of treatment response and secondly, since the volume of intraepithelial tumor cells is relatively small, gene therapy for pre-cancer would require eliminating smaller numbers of tumor cells as compared to the advanced stage of cancer. Thirdly, due to the bystander effect, not all premalignant cells would need to express the suicide gene for therapy to be effective. In suicide gene therapy, the bystander effect refers to the capacity of transfected cells to transfer death signals to the neighbouring tumor cells. In a study conducted by Sandalon *et al.* (2001)^[33], the efficacy of suicide gene therapy was studied in a organotypic tissue model of premalignant disease using the suicide gene cytosine deaminase (CD). CD encodes a bacterial enzyme that can convert non-toxic 5-fluorocytosine (5FC) to the toxic anabolite 5-fluorouracil (5FU), which results in the death of the genetically modified cells. The study revealed that a significant number of intraepithelial cells with malignant potential could be eliminated^[33].

Previous studies reported have shown that there is abnormal p53 expression in almost 20 percent of the patients with oral leukoplakia and the mutations in the p53 gene and alterations in the p53 protein result in its accumulation in cells that may play a crucial role in tumorigenesis^[34]. Transduction of the wt-p53 gene into oral leukoplakia cells restores the tumor suppressor functions and prevents the cancer progression. With recombinant human adenovirus-p53 (rAd-p53), a replication in competent human type 5 adenovirus in which the E1 region has been replaced with an expression cassette containing the human wt-p53 cDNA, this transduction of wt-p53 has been accomplished. In a study conducted by Li *et al.*, intraepithelial injections of recombinant adenovirus-p53 were introduced in twenty two patients with dysplastic oral leukoplakia. After completion of this course of treatment and after 24 months of follow-up, it was observed that 5 cases experienced a complete regression while 20-70% areas of the lesion disappeared in 11 cases^[17]. The hypothesis that even in the precancerous state, there are p53 cascade inactivating mutations that will allow for the replication of oncolytic adenovirus and eventually the rapid elimination of the cells before they turn malignant has led to the testing of ONYX-015 for the preventive treatment of oral precancer. Also, ONYX-015 has been found to be selective in its cytotoxic property towards cells with defects in p53 dependent response pathways as compared to isotretinoin, which is one of the most commonly used chemopreventive agent. Finally, ONYX-015 may reveal potential synergism with retinoid therapy for oral cancer chemoprevention^[35]. These researches provide a brand new way to approach a potentially malignant disease.

CONCLUSION

Oral squamous cell carcinoma is a major health problem especially in developing countries where awareness is low and is associated

with significant morbidity and mortality. Surgery, chemotherapy and radiotherapy are the mainstay of treatment protocol for oral squamous cell carcinoma that are associated with various adverse effects. Most of the oral squamous cell carcinoma cases usually are preceded by potentially malignant lesions and early diagnosis and treatment can be an important intervention strategy to prevent their progression to advanced stages of invasive oral carcinoma.

In the fast paced growth of medical technology where in there is a surge of newer therapeutic strategies and a better understanding of the molecular mechanisms involved in cancer, it is prudent to discuss the relevance of gene therapy as an important modality. Gene therapy can be applied for the treatment of advanced oral squamous cell carcinoma to prevent adverse side effects of currently available treatment methods so as to enable a better quality of life and also as an important aid to prevent malignant transformation of precursor lesions of the oral cavity.

Cancer gene therapy is now moving from the initial clinical trial level to the next level. With these various clinical trials being carried out to test the efficacy of the gene therapy approaches there is lack of sufficient evidence to support the effectiveness of this modality as a definitive treatment for head and neck cancer. Some of the barriers to the success of such a therapy that need to be taken care of are optimization of vectors that can transfer DNA to the target cells, diminish the viral vectors associated biological risks like toxicity and immunogenicity, prevention of undesirable heterologous gene or vector expression^[4,5]. However continued diligent research efforts to combine gene therapy with the pre-existing treatment protocol such as chemotherapy, radiotherapy and surgery is the need of the hour. It is hence necessary to understand and further the use of gene therapy in head and neck cancer in order to benefit the vast number of people worldwide suffering from this dreadful disease.

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

REFERENCES

1. Ferlay J, Shin HR, Bray F. Estimates of worldwide burden of cancer in 2008: GLOBOCON 2008. *Int J Cancer* 2010; **127**: 2893-2917.
2. Jemal A, Siegel R, Xu J. Cancer statistics, 2010. *Ca Cancer J Clin* 2010; **60**: 277-300.
3. Rivera C, Venegas B. Histological and molecular aspects of oral squamous cell carcinoma (Review). *Oncology Letters* 2014; **8**: 7-11.
4. Amer MH. Gene therapy for cancer: present status and future perspective. *Molcellther* 2014; **2**: 1-19
5. Freire JE, Medeiros SC, Neto AV, Junior JE, Sousa AJ, Rocha AJ. Bioethical conflicts of gene therapy: a brief critical review. *Rev Assoc Med Bras* 2014; **60**: 520-4.
6. Cotrim AP, Baum BJ. Gene therapy: some history, applications, problems and prospects. *ToxicolPathol* 2008; **36**: 97-103.
7. Cox DBT, Platt RJ, Zhang F. Therapeutic genome editing: prospects and challenges. *Nature medicine* 2015; **21**: 121-131.
8. Zhang C, Wang QT, Liu HE, Zhang ZZ, Huang WL. Advancements and prospects of tumour gene therapy. *Chin J Cancer* 2011; **30**: 182-188.
9. Scanlon KJ. Cancer gene therapy: challenges and opportunities. *Anticancer Res* 2004; **24**: 501-504.
10. Chodisetty S, Nelson EJ. Gene therapy in India: A focus. *JBiosci* 2014; **39**: 537-541.

11. Baum BJ. Gene Therapy. *Oral Dis* 2014; **20**: 115-118.
 12. Mitrovic T, Radulovic S. Cancer gene therapy. *Arch Oncol* 2005; **13**: 23-26.
 13. Mathis JM, Stoff-Khalili MA, Curiel DT. Oncolytic adenoviruses - selective retargeting to tumor cells. *Oncogene* 2005; **24**: 7775-7791
 14. Bergen M, Chen R, Gonzalez R. Efficacy and safety of HLA-B7 / beta 2 microglobulin plasmid DNA/lipid complex (Allovecin 7) in patients with metastatic melanoma [J]. *Expert Opin Biol Ther* 2003; **3**: 377-384.
 15. Ganci F, Sacconi A, Manciocco V, Covello R, Spriano G, Fontemaggi G et al. Molecular Genetics and Biology of Head and Neck Squamous Cell Carcinoma: Implications for Diagnosis, Prognosis and Treatment. *Head and Neck Cancer* 2012; 73-122.
 16. Williams HK. Molecular pathogenesis of oral squamous carcinoma. *J Clin Pathol: Mol Pathol* 2000; **53**: 165-172.
 17. Li Y, Li LJ, Zhang ST, Wang LJ, Zhang Z, Gao N et al. In vitro and Clinical Studies of Gene Therapy with Recombinant Human Adenovirus-p53 Injection for Oral Leukoplakia. *Clin Cancer Res* 2009; **15**: 6724-6731.
 18. Cross D, Burmester JK. Gene therapy for cancer treatment- past, present & future. *Clin Med Res* 2006; **4**: 218-227.
 19. Tysome JR, Lemoine NR, Wang Y. Update on oncolytic viral therapy – targeting angiogenesis. *Onco Targets Ther* 2013; **6**: 1031-1040.
 20. Thomas SM, Grandis JR. The current state of head and neck cancer gene therapy. *Hum Gene Ther* 2009; **20**: 1565-1575.
 21. Nemunaitis J, Ganly I, Khuri F, Arseneau J, Kuhn J, McCarty T, et al. Selective replication and oncolysis in p53 mutant tumors with ONYX-015, an E1B-55kD gene-deleted adenovirus, in patients with advanced head and neck cancer: A phase II trial. *Cancer Res* 2000; **60**: 6359-6366.
 22. LumnitzkyK, Safrany G. Cancer gene therapy: combination with radiation therapy and the role of bystander cell killing in the anti-tumor effect. *Pathol Oncol Res* 2006; **12**: 118-124.
 23. Liu TJ, Zhang WW, Taylor DL, Roth JA, Goepfert H, Clayman GL. Growth suppression of human head and neck cancer cells by the introduction of a wild-type p53 gene via a recombinant adenovirus. *Cancer Res* 1994; **54**: 3662-3667.
 24. Li Y, Li LJ, Wang LJ, Zhang Z, Gao N, Liang CY, Huang YD et al. Selective intra-arterial infusion of rAd-p53 with chemotherapy for advanced oral cancer: a randomized clinical trial. *BMC Med* 2014; **30**: 12-16.
 25. Barbellido SA, Trapero JC, Sanchez JC, Garcia MP, Castano NE, Martinez AB. Gene therapy in the management of oral cancer: Review of the literature. *Med Oral Patol Oral Cir Bucal* 2008; **13**: E15-21.
 26. Yi Y, Noh MJ, Lee KH. Current advances in retroviral gene therapy. *Curr Gene Ther* 2011; **11**: 218-228.
 27. Shillitoe EJ. Gene therapy: the end of the rainbow? *Head neck Oncol* 2009; **1**: 1-5
 28. Simmons RK, Baum BJ. Transferring Genes to Salivary Glands. *J Dent Educ* 2001; **65**: 907-910
 29. Yamano S, Baum BJ. Prospects for gene-based immunopharmacology in salivary glands. *Jpn J Pharmacol* 2000; **82**: 281-286.
 30. ReibelJ. Prognosis of oral pre-malignant lesions: significance of clinical, histopathological, and molecular biological characteristics. *Crit Rev Oral Biol Med* 2003; **14**: 47-62.
 31. Gonzalez-Moles MA, Scully C, Ruiz- Avila I. Molecular findings in oral premalignant fields: update on their diagnostic and clinical implications. *Oral Dis* 2012; **18**: 40-47.
 32. Mortazavi H, Baharvand M, Mehdipour M. Oral potentially malignant disorders: An overview of more than 20 entities. *J Dent Res Dent Clin Dent Prospect* 2014; **8**: 6-14.
 33. Sandalon Z, Fusenig NE, McCutcheon J, Taichman LB, Garlick JA. Suicide gene therapy for premalignant disease: a new strategy for the treatment of intraepithelial neoplasia. *Gene Therapy* 2001; **8**: 232-238
 34. Shin DM, Mao L, Papadimitrakopoulou VM, Clayman G, El-Naggar A, Shin HJ et al. Biochemopreventive Therapy for Patients With Premalignant Lesions of the Head and Neck and p53 Gene Expression. *J Natl Cancer Inst* 2000; **92**: 69-73
 35. Rudin CM, Cohen EE, Papadimitrakopoulou VA, Silverman S Jr, Recant W, El-Naggar AK. An attenuated adenovirus, ONYX-015, as mouthwash therapy for premalignant oral dysplasia. *J Clin Oncol* 2003; **21**: 4546-52.
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