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A Systematic Review of Gene Therapy as Treatment of Oral Squamous Cell Carcinoma

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) represents about 90% of all malignant neoplasms of the oral cavity. Oral cancer is the cause of death among children ages 1-14 years. Gene therapy provides modern medicine and had great potential as a recent therapeutic modality. Gene therapy can potentially attack cancerous cells while respecting normal tissue. It may be useful as treatment for recurrence disease such as oral squamous cell carcinoma.
Objective: To review materials about gene therapy as a treatment for oral squamous cell carcinoma.
Methods: Scientific evidence and clinical cases were drawn from the literature to support this review and information about the gene therapy as a treatment of oral squamous cell carcinoma was collected.
Results: Gene therapy is introducing new genetic material into target 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. Gene therapy includes both the transfer of new genetic material and the manipulation of existing genetic material.
Conclusion: Gene therapy has potential as a recent treatment in cases of oral squamous cell carcinoma (OSCC).
Keywords: Gene therapy, Oral squamous cell carcinoma (OSCC)
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INTRODUCTION

Oral squamous cell carcinoma represents about 90 of all malignant neoplasms of the oral cavity in human.[1] Oral cancer is the 6th most common malignancy worldwide. Patients passed away because of cancer of the oral cavity and lip as much one hundred and forty five thousand patients.[2] According to histological data, oral squamous cell carcinoma accounts for majority of all oral cancer in the world.[3] Oral squamous cell carcinoma is rare in pediatric patients which defined by the American Academy of Pediatrics as patients under age 21. Many clinicians believe that this disease is associated with poorer survival compared to adults.[4]

The most risk factors of oral squamous cell carcinoma (OSCC) are tobacco and alcohol abuse. According to the International Agency for Research on Cancer that cigarettes smoke contains more than 60 chemical carcinogens.[2] Alteration of taste, pain, difficulty in eating, bacterial and fungal infections, mucosal ulceration, increased thickness of saliva, discoloration skin, and edema of the skin are the major side effects.[5,6]

Gene therapy provides modern medicine and had great potential as a recent therapeutic modality. Clinical application of this technique in the treatment of oral cancer and precancer requires optimization of viral vectors and improvement of transfection effectiveness.[7,8,9] Here we use systematic review and meta-analysis to describe more deeply about gene therapy as a treatment of oral squamous cell carcinoma.

Oral Squamous Cell Carcinoma (OSCC)

Oral cancer is the 6th most common malignancy worldwide. Patients passed away from cancer of the oral cavity and lip as much one hundred and forty five thousand.[2,4] The etiology of oral squamous cell carcinoma there are:

a. Tobacco

More than 80% consumption of tobacco with smoking greatly increases the risks of oral squamous cell carcinoma. The strongest being tobacco-specific N-nitrosamines is several carcinogens in tobacco.[10,11,12]

b. Alcohol

The combined use of alcohol and tobacco has a multiplicative effect on oral cancer risk. The various pathways by which alcohol may exert carcinogenic influence include topical exposure leading to a direct effect on cell membranes.[10,13]

c. Genetic

Cytochrome p450 system is most carcinogens which metabolized in the liver. Individuals with the fast metabolizing version of alcohol dehydrogenase have a greater risk of developing oral cancer.[10,14,15]

Potential symptoms of the oral squamous cell carcinoma are:[3]

a. Non healing ulcer with or without induration
b. White patch with firm consistency

c. Red lesion or with erythroplasia
d. Abnormal lump in the mouth
e. Exophytics proliferative growth
f. Lymph node enlargement

The mechanism of pathogenesis of oral squamous cell carcinoma there is genetic alterations include activation of oncogenes that promote proliferation, as well as inactivation of TSGs. In oral dysplastic lesions and oral squamous cell carcinoma, the increased expression of antiapoptotic proteins either Bcl-2 has been detected.[16,17,18,19,20]

Managements for oral squamous cell carcinoma are:[21,22,23,24,25]

a. Chemoradiotherapy

The first line of treatment of oral squamous cell carcinoma is chemotherapy using drugs like cisplatin, 5-fluorouracil, and docetaxel. Chemotherapy is effective in oral carcinoma when used in combination.

b. Surgical Interventions

Surgical removal of the tumor is done if the tumor is small enough and if surgery is likely to result in functionally satisfactory results. When a tumor is inoperable, chemoradiotherapy is often used with surgery. After the surgery, most oral cancer patients, depend on a feeding tube for their nutrition.

c. COX-2 Inhibitors

Cyclooxygenases (COX) are rate limiting enzymes as catalysts in the synthesis of prostaglandins from arachidonic acid. COX-2 is an inducible enzyme that is elevated during inflammation.

d. Photodynamic Therapy

Several small clinical studies have shown that photodynamic therapy is effective in treating premalignant lesions and superficial carcinoma. Hematoporphyrin derivatives and its conjugates are administered either topically or by intravenous route.

Gene Therapy

Gene therapy is a gene transfer for treating human diseases, which includes both manipulation of the existing genetic material and the transfer of new genetic material. The first successful treatment was of X-linked Severe Combined Immunodeficiency (X-SCID) by ex vivo gene replacement therapy.[7,26]

The purpose of gene therapy is introducing new genetic material cancerous cells while causing no damage to surrounding healthy cells and tissue. At the present time, the most widely used gene therapy procedure follows these steps:[7,27,28]

1) Identification, isolation and amplification of the gene to be used in the treatment for patients.
2) Extraction and in vitro culture of tissue cells from the patient to be treated.
3) Transfer of the therapeutic gene into these cells via a vector, using a gene that contains a promoting sequence to enable its expression and a marker to identify cells.
4) Transfer into the patient of selected gene containing cells. The theory is that when the gene exerts its normal physiological functions.

The main purpose of gene therapy is to introduce new genetic material into the target cells without causing of damage to the surrounding normal cells. The technique of gene therapies are:[28,29,30,31]

A. Gene Excision Therapy

In this technique, the defective oncogenes are removed, as a result of which, there is an inhibition in the growth of the tumour cells.

B. Gene Addition Therapy

Genetic alterations include mutations of p53, the retinoblastoma gene, p21, and p16. Tumour suppressor gene which is commonly used in gene therapy is the p53 gene and 60% of the human tumours are associated with mutations at the p53 locus.

C. Antisense RNA Therapy

The Antisense RNA checks the tumour growth by inhibiting the RNA which is complementary to the strands of the DNA which expresses that particular gene.

D. Suicide Gene Therapy

Many studies have been done on the gene delivery system with retrovirusor adenovirus vectors. This therapy involves enzymes, the expression of which transforms the non-toxic producing drug into an active cytotoxic substance.

Advantages of gene therapy are:[9,32,33]

1. Functional gene can replace a defective gene.
2. Aids in the prevention of potentially toxic effects in the body.
3. It decreases the cost of various therapies and improves the patient's lifestyle for a longer period.

METHODS

Literature Search and Research Methods

Relevant full publications and meeting abstracts were identified by electronic searching of online databases (ClinicalTrials.gov, National Library of Medicine) using the search term: <gene therapy> AND <oral squamous cell carcinoma>.[16] The methods of this research are:[17]

Cell Culture

This study used the TCA8113 (tongue squamous cell carcinoma), MG63 (osteosarcoma), Eca-109 (esophageal cancer), HeLa (endocervical adenocarcinoma), MCF-7 (breast cancer) human cancer cell lines, and the L02 (spontaneously immortalized hepatic cells) normal cell line.

Real-Time Fluorescence Quantitative PCR

Total RNA was isolated from cells according to the instructions of a TaKaRa Mini BEST Universal RNA Extraction Kit. The amplification was monitored on an ABI Prism 7500 real-time PCR apparatus (Applied Biosystems) using SYBR Green detection chemistry (TaKaRa).

Cells Early Apoptotic Changes Detected by TUNEL Assay
The cells were cultured and transfected as described above. Afterward, the cells were collected after plasmid transfection and evaluated using a TUNEL cell apoptosis assay kit (Biyuntian Biotechnology Co., Ltd.).

Data Extraction and Analysis
Statistical analyses were performed using GraphPad Prism 5.0. All data were expressed as the mean SEM. Differences with *P* values <0.05 were considered significant.

RESULTS

Expression of the SERPINB3 Gene in Different Human Cell Lines Determined by Western Blotting and RT-qPCR
To determine the potential of the SERPINB3 gene for the targeted therapy of squamous cell carcinoma, we detected the expression of the SERPINB3 gene in different cell lines (TCA8113, HeLa, MG63, L02) by Western blotting and RT-qPCR.[17]

Inhibition of Protein Synthesis Induced by the pSERPIBN3-PE38KDEL Plasmid
These results showed that among the plasmids tested, the recombinant toxic gene expression plasmid pSERPINB3-PE38KDEL inhibited the expression of luciferase in TCA8113 cells most strongly, which indicated that the plasmid strongly inhibits protein synthesis in TCA8113 cells.[17] (Figure 1).

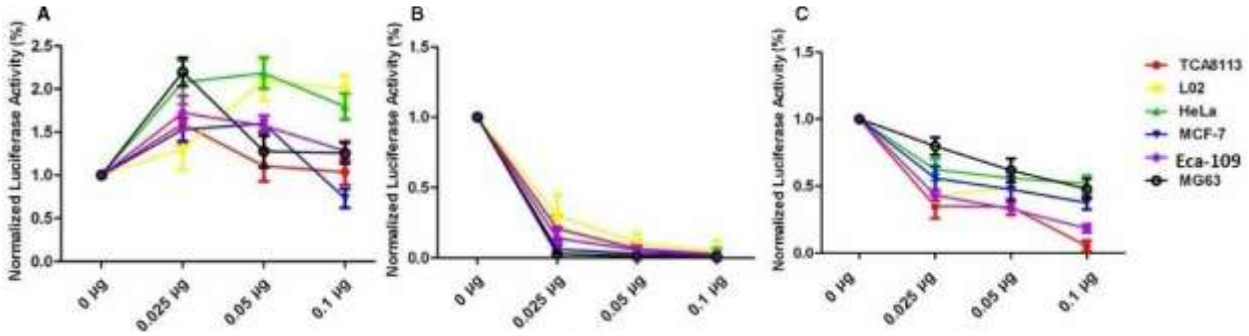

Figure 1: Inhibition of protein synthesis induced by the pSERPIBN3-PE38KDEL plasmid. A) After 48 h of cotransfected with different quantities (0 μg, 0.025 μg, 0.05 μg, or 0.1 μg) of the negative control plasmid pGL3-Basic. B) After 48 h of cotransfected with different quantities (0 μg, 0.025 μg, 0.05 μg, or 0.1 μg) of the positive control plasmid pcDNA-PE38KDEL C) After 48 h of cotransfected with different quantities of the pSERPINB3-PE38KDEL plasmid (0 μg, 0.025 μg, 0.05 μg, or 0.1 μg).

(Source: Wu J, Guo Q, Zhang G, et al. (2020). Study on the targeted therapy of oral squamous cell carcinoma with a plasmid expressing PE38KDEL toxin under control of the SERPINB3 promoter. *Cancer Med*. 00:1–10

Early Apoptosis Analysis by TUNEL Assay
These results showed that the rate of TCA8113 cell apoptotic necrosis was significantly higher than that of MG63 and L02 cells after 48 hours of transfection.[17]

DISCUSSION

Today, the research on gene therapy in oral cancer is increasing in the laboratory and the clinical settings. Gene therapy is now moving from phase 1 and 2 trials to the next level. Considerable time and a large number of patients are required to demonstrate the true efficacy of the therapies.[7,34] Combination gene therapy which uses several genes showed significant tumor regression in animal studies. The results showed a low toxicity and high efficacy.[35,36,37,38]

In India, Advanced Centre for Treatment, Research and Education for Cancer (ACTREC, Mumbai) was the 1st Centre dedicated to gene therapy research. Rita M ulherkar's group from ACTREC is conducting gene therapy studies related to the treatment of HNC using viral vectors.[9] The clinical application of gene therapy for treatment of oral cancer will require optimization of gene delivery.[39,40,41,42]

Phase II clinical studies by various investigators have showed promising results when gene therapy combined with chemotherapy, surgery, or radiation therapy. Such augmentative approaches, will reduce morbidity of the treatment and help maintain quality of life in patients with oral squamous cell carcinoma.[43,44,45]

As shown in this review, research on gene therapy in oral cancer is increasing in the laboratory and in the clinical settings. 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.[46]

In the 1970, Kato and Torigoe isolated squamous cell carcinoma specific antigen from cervical squamous cell carcinoma. The antigen is composed of two isomers with almost the same molecular weight, SERPINB3 and SERPINB4, among which SERPINB3 is a serine protease inhibitor. The Western blotting and RT-qPCR results in this study proved that the SERPINB3 gene was expressed.[17,47]

According to research conducted by U.S. National Library of Medicine, some cancers that occur in the mouth may in some way be due to a defect in a gene called the p53 gene. The parts of the adenovirus that allow it to reproduce and promote infection are removed, and the experimental gene (p53) is added.[48]

The mouth rinse will be given one time on the first day and two times on days 2 until 5. The injection and rinse (first day of each cycle), or the two rinses (days 2 - 5 of each cycle), will be separated by at least two hours. Eating and drinking should be avoided for one hour after receiving the mouth rinse. The injection and rinse series will be repeated on a monthly basis for a period of six months.[48,49]

Participants will be requested to have two biopsies (small tissue samples) of the premalignant lesion taken on day 5 of the 1st and 6th courses. These studies will also determine if the gene therapy injections have been effective in delivering the gene (p53) to the precancerous cells.[48]

Gene transfer, while a radical new type of treatment, is also the only gene therapy product to obtain regulatory approval in any global market, as demonstrated by China's 2003 approval of Gendicine for clinical use. Gendicine is a modified adenovirus that delivers the p53 gene to cancer cells and is approved for the treatment of head and neck squamous cell carcinoma.[50]

Gene transfer technology allows an incredible diversity of treatment possibilities. New delivery methods and more sophisticated gene expression cassettes will create better therapeutic alternatives to make the goal of cancer treatment and eradication achievable.[50,51,52,53]

CONCLUSION

Gene therapy has potential as a treatment in cases of oral squamous cell carcinoma (OSCC).

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