

Research Article

Gene Therapy Trail for Treatment of Tumor in Pregnant and Non Pregnant Mice by Mixed DNA with RNA

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Abstract: This study was conducted on 35 Adult Mus Musculus (Swiss albino) mice, it was suffering from tumors (parotid gland adenocarcinoma and another subcutaneous tumor). The mice divided in two groups, 25 mice treated and 10 mice control group. Total genome extracted from liver of camel by lysis buffer solution (100 mM NaCl, 1 mM EDTA, 10 mM Tris-HCl pH 8.0) 5% SDS and purification by chloroform-phenol. Treated group subjected for gene therapy by in situ injection of 0.1cc mixed camel nucleic acid three times, until tumor mass disappear. All mice for control group were dead, while the mice treated still alive. In conclusion, the total genome of camel may play an important role in the defense mechanism of the body against adinoarcinoma.

Keywords: Gene therapy, pregnant mice, tumor

INTRODUCTION

A salivary gland Adenocarcinoma in a white Swiss mouse originated from the serous cells of the parotid salivary gland. Retroviral particles were detected in the neoplastic salivary gland cells by electron microscopy (Bundza *et al.*, 1989). Gene therapy can be defined as the introduction of nucleic acids into cells for the purpose of altering the course of a medical condition or disease. Many types of parotid malignancies exist, most arising from the epithelial elements of the gland. Classification of these tumors can be quite confusing. In addition, malignancy may develop in the secretory element of the gland or malignancy arising elsewhere may first be noticed as a metastasis to the gland (Spiro, 1986; Stafford and Wilde, 1997). Therefore, if the primary tumor already necessitates adjunctive radiation therapy then it seems reasonable to use radiation to treat the neck as well (Regis *et al.*, 2001; Kelly and Spiro, 1996). Radiation to the neck increases the risk of malignancy of salivary glands with a 15- to 20-year latency (Schneider and Sarne, 2005).

Some studies have suggested an association between high use of mobile phones and an increased risk of benign and malignant parotid tumours (Sadetzki *et al.*, 2008). There are two major classes of vehicles for gene transfer: viral and nonviral vectors (Kay *et al.*, 1997). A vector should also be able to express the gene for as long as it is required, generally the life of the patient (Crystal, 1996). A normal copy of the gene to restore the function of the protein. A gene that is inserted directly into a cell usually does not function. Instead, a carrier called a vector is genetically engineered to deliver the gene. Certain viruses are often used as vectors because they can deliver the new gene

by infecting the cell. The viruses are modified so they can't cause disease when used in people. Some types of virus, such as retroviruses, integrate their genetic material (including the new gene) into a chromosome in the human cell. Other viruses, such as adenoviruses, introduce their DNA into the nucleus of the cell, but the DNA is nonintegrated into a chromosome (http, 2012). Enhancing the immunogenicity of the tumor, for example by introducing genes that encode foreign antigens (Satoh, 2005). The vector that replicates within cancer cells, causing cellular death.

Therefore, this study concentrated on the treatment of tumor by gene therapy with camel nucleic acid in situ injection of tumor.

MATERIALS AND METHODS

The study was used 35 adult Mus Musculus (Swiss albino) mice, it was suffered from salivary gland adinoarcinoma. A swelling of the left side of the neck and abdominal, with the right side of the neck (Fig. 1a to d), characterized by firm mass, size ranged from (1-3.5 cm). Salivary Duct Carcinoma (SDC) is a high-grade neoplasm known to histological resembles high-grade ductal carcinoma in situ of the breast (Henley *et al.*, 2000). Gross sections were grayish with cystic small areas. The mice was divided in two groups, the first 25 mice were subjected to injection of total extracted genome of camel, by needle gage 23. Total genome were extracted from liver of healthy camel by using lyses buffer, consist of (100 mM NaCl, 1mM EDTA, 10 mM Tris-HCl pH 8.0), dissolved in 100 mL distil water, sterilized by autoclaving 15 min, added 0.5% SDS in 55°C. Stored in refrigerator (4°C) and purification by chloroform-phenol, precipitating by



Fig. 1: Show the different site of tumor, a) salivary gland, b) behind forelimbs, c) abdominal subcutaneous and d) salivary gland

absolute ethanol and running in the Gel electrophoresis (Sambrook *et al.*, 1989). Second group (10) mice represented the control group injected by normal saline. Tumor specimens obtained were fixed in 10% neutral buffered formalin, embedded in paraffin, cut at 5- μ m thickness and stained routinely with hematoxylin and eosin for histological examination. During treatment, females leaved with the males for conception. Treated mice were subjected to electrical current (3 volt) immediately.

RESULTLS AND DISCUSSION

Total DNA extracted mixed with RNA (Fig. 2) with running by gel electrophoresis (Fig. 3). We observed the first group of infected mice, still alive and complete recovery after treatment, females fertilized by male through the period of treatment, pregnant and then normal delivery. All mice in the second group (control) was dead in variable periods, ranged between 2 to 8 weeks, depended the site of tumor. Histological the tumor appeared cellular proliferative activity was shown mitotic finger (Fig. 4), the nuclei were hyperchromatic and cystic dilatation architecture, Papillary growth pattern, some infiltrated by WBCs and congestion of blood vessels (Fig. 5).

Until recently, neoplasm still one of the first problem in the world. These study trials for tumor treatment by used the (camel genome) from the genus *Camelus dromedaries*. Nucleic acid direct injected into affected organ. Gene therapy is an experimental treatment that involves introducing genetic material (DNA or RNA) into cells (Moghaddam and Antonio Chiocca, 2009). Treated group increased activity, reduction of tumor and reached the half in size, finely the tumor disappeared and the animals still alive. Previously, the control group gives normal activity subsequent with the advanced period of study, the tumor more enlargement, heirless locally, dullness, weakness, emaciation with the drop and dead eventually. Total genome (DNA, RNA) may play an important role in the defense mechanism of the body against adinoarcinoma, or may be some expression of RNA. Somatic gene therapies are in clinical trials, targeting several different tissues, gene delivery may be directly to the affected tissue, or into cells that can produce the needed protein (Lewis, 2007). We are used this method in order to avoid the complication of vector like viral DNA expression or any vector types. After the genes are injected into the region, there is the



Fig. 2: Mixed DNA & RNA separated by phenol-chloroform



Fig. 3: Camel chromosomal DNA bands on 1% agarose gel after 30 min electrophoresis at 50 volt

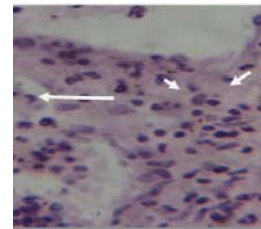


Fig. 4: Adenocarcinoma show the mitotic figure in epithelium of parotid gland. Hematoxylin and eosin



Fig. 5: Papillary growth pattern

possibility that the viral vectors that are used to transfer the genetic material into the cell might infect healthy cells as well as cancer cells (Trinh, 2005). There are several ways in which researchers are using gene therapy to treat cancer cells. In one approach, researchers add healthy functioning genes to cells that have damage or missing genes in order to substitute the bad non-working copy of the gene with a better copy of

the working gene (Trinh, 2005). Histological, epithelial cells, are large and polygonal in shape and they display a finely granular, vacuolated the cell borders are well defined. The nuclei are round to oval in shape, hyperchromatic and moderately pleomorphic. Conspicuous mitotic figures are seen. The presence of intermediate cells and mucous cells, however, argues against SDC (John *et al.*, 2000). The result display the control group were dead, while the treated group gives resistant. Treated mice were subjected to electrical current (3 volt) immediately, for stimulated cells with gene therapy. Electro-transfer, on the other hand, is being developed for the delivery of oligonucleotides or custom designed plasmids. Short pulses of relatively high-intensity electric fields are used for electro-transfer delivery, nonviral delivery systems are safe and can be used efficiently for targeted gene delivery to ocular tissues in vivo (Bejjani *et al.*, 2007) Camel genome used because it has nanobodies. Nanobodies produced by the blood of camels have unique properties that can be used for the development of future drugs (Admin, 2011). Another that varies study mention the effect of urine of camel against the tumor therefore the genome of camel it's the source of the cytotoxic materials of cancers cells. Camel urine showed cytotoxicity against various, but not all, human cancer cell lines, with only marginal effect on non-tumorigenic epithelial and normal fibroblast cells epithelial and fibroblast cells (Al-Yousef *et al.*, 2012).

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