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Gene therapy in head and neck cancer

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Abstract

Gene therapy using recombinant DNA a new method that utilises technology to revolutionise treating genetic and acquired diseases. This method involves the modification or replacement of defective genes to correct or alleviate disease symptoms. Vectors, and especially viral vectors, have come a long way in the last few decades; they transport therapeutic genes to their intended targets in cells. Recent developments in gene-editing technologies, such as CRISPR-Cas9, have further accelerated this transformation by making it possible to make exact changes to genetic code with minimal off-target effects. Despite these advancements, gene therapy faces several challenges. Immune responses to viral vectors, the potential for insertional mutagenesis, and difficulties in delivering the therapy to specific tissues are major hurdles that need to be addressed. Moreover, the high cost of gene therapy and ethical concerns surrounding genetic modifications continue to spark debate.

Keywords: Gene therapy, cancer, off-target effects, DNA

Introduction

Head and neck malignancies are often treated with surgery, radiation treatment, and chemotherapy. Patients diagnosed with head and neck cancer still do not have much better survival rates by these therapy approaches. Around one-third of individuals have local and/or regional recurrence even after definitive therapy. It is common to think of recurrence as incurable in people whose cancer has extended to several regions of the physique. The margins of very big tumors may be difficult to remove with conventional treatment, and some cancers are exceptionally resistant to chemotherapy and radiation. Increasing the dosage of radiation or chemotherapeutic agent results in an unacceptable level of toxicity and collateral harm to healthy tissue. Even while there has been some success with combining existing therapy methods, the toxicity that results from these combination medicines is typically intolerable, and patients still don't have a better chance of survival. The toxicity to healthy organs and lack of tumor cell selectivity are the main problems with this traditional therapy.

New evidence from molecular biology has linked gene therapy to tumor development. There is hope that gene therapy may selectively target cancer cells while avoiding harming healthy cells. Optimizing gene delivery and determining transfection efficiency are prerequisites for clinically using gene therapy to treat head and neck cancer. When used to treat human illness, gene therapy entails both incorporating novel genomic sequences and modification of existing genetic material. When dealing with cancer, gene therapy allows for the targeting of oncogenes while sparing healthy cells. This article's goal is to provide a synopsis of where gene therapy stands in relation to head and neck cancer. The larynx, oral cavity, oropharynx, and hypopharynx are all potential sites for tumour formation in head and neck squamous cell carcinoma (HNSCC), one of the most prevalent types of cancer.

Literature Review

There is a new field of medicine called gene therapy. Immunodeficiency, a rare neurotransmitter disorder, retinal dystrophy, spinal muscular atrophy, haemophilia, and specific haematological malignancies were the first indications for our medications, and we are now evaluating a plethora of other indications in research studies. By outlining the fundamentals of gene therapy and discussing its potential benefits and drawbacks, this review hopes to serve as a useful resource for anybody interested in the field. Using hemophilia and spinal muscular atrophy as examples,

the possible benefits, negative consequences, and hazards are shown. Gene augmentation, which involves adding genes either *in vitro* or *in vivo*, is now the most well-established kind of gene therapy. The spotlight has now shifted to more nuanced and exact methods, such in situ gene editing. Yet, in order to guarantee safety, effectiveness, predictability, and durability, all gene therapies need long-term monitoring of individuals who have been treated. Some major safety issues include on immunological reactions to the vector, foreign DNA or proteins produced by gene therapy, and the fact that insertional mutagenesis maintains a low cancer risk. While this innovative therapy places a financial strain on the health care system, new compensation methods are required to alleviate some of the ethical and regulatory concerns that have arisen. The therapeutic implications of gene therapy for hereditary disorders are substantial, but the potential for significant improvement or perhaps cure is enormous. Still, there are a lot of things that require addressing before people may have easy access to safe and effective goods.

Some hereditary diseases advance to the point that organ failure or death occurs. Different inheritance mechanisms, gene types, and population-based differences make it challenging to discover viable solutions for almost 400 million people globally, and there are no permanent therapeutic options for over 95% of hereditary conditions. Restoring, replacing, inhibiting, or altering genes to recover the illness phenotype is the goal of one possible molecular strategy for treating rare hereditary disorders is gene therapy. Lentivirus and adeno-associated virus (AAV) are two examples of the rapidly improving viral vector systems that are now being used in 64.2% of gene therapy clinical studies, according to current data. Clinical trials involving the eye, blood, and neuro-muscular systems have shown tremendous potential in the use of AAV-mediated gene therapy for the treatment of inherited disorders. Prior to being authorised for use in clinical and pre-clinical studies, vectors must demonstrate both safety and effectiveness. A lot of money, a lot of new information, and a lot of technology go into making clinical-grade vectors, testing them, and getting them approved for use in gene therapy. Every dosage of a gene therapy product costs an arm and a leg because of the high need for educated labor to keep up with the rapid pace of technological advancement. Historically, the Indian subcontinent has been behind other regions when it comes to gene therapy clinical trials. This is because of a lack of resources, an outdated scientific and industrial infrastructure, and inaccessible patient databases. Nonetheless, progress in India has been accelerated by the growing global recognition of rare illnesses and the approval of gene treatments on an international scale.

Behl, Tapan *et al*. (2020) [1]. Alternative new methods for treating genetic disorders have emerged from the limitations of standard therapies for Parkinson's illness. The regeneration of dopaminergic neurons, enzyme synthesis, neurons in the subthalamic nucleus, modulation of astrocyte neurotrophic factors, microglial cells, and are all topics covered by these therapies. Prodrug methods, dopamine synthesizing enzyme encoding gene delivery, fetal ventral mesencephalon tissue transplantation, viral vector-based dopamine administration, and other noteworthy treatments have been extensively tested and shown to be effective. This review focuses on the function of GDNF, which is generated from glial cell lines, in reducing motor symptoms and the degeneration of dopaminergic neurons in Parkinson's disease. Ret receptor of tyrosine kinase family activation occurs only in dopaminergic neurons upon binding of GDNF family ligands to related receptors. Infusion catheter-assisted intraparenchymal and intraluminal delivery, as well as ventricular delivery, are included in the review. Gene therapy combinations, delivery vector optimization, improved targeting devices, and delivery systems based on liposomes and encapsulated cells are some of the suggested ways to overcome obstacles.

Pawar, Aakash *et al*. (2023) [2]. Based on biotechnology, recombinant DNA (RDT) generates two or more DNA molecules in a single molecule. This dynamic area is quickly expanding as it merges biological and technological aspects. Because it aids in the fight against human illnesses, RDT is crucial in the biotechnology and pharmaceutical industries. Bioremediation, agriculture, medicine, health, and the environment are some of its possible uses. RDT is used to manufacture insulin for humans and is essential in the creation of vaccinations against viruses, bacteria, and protozoa. In addition to its critical function in crop development, RDT has a major influence on the production of biopharmaceuticals and agriculture. Scientists all across the globe are rethinking RDT's methodology, which is opening up new areas of study and ultimately contributing to the development of biology.

Wang, Xiao-Yu *et al*. (2022) [3]. Gene therapy makes extensive use of LVs, or lentiviral vectors, are a kind of viral vector that originate from the HIV virus. The last 20 years have seen LV undergo steady improvement in terms of transduction efficiency, safety, and selective targeting. Multiple plasmids containing the viral genome have been isolated, and any unnecessary gene elements have been deleted. To improve the effectiveness of tissue targeting and transduction, several viral envelope proteins and posttranscriptional regulatory components have been included. LV has found extensive usage in gene therapy for the treatment of neurological disorders, immunodeficiency disorders, hemoglobinopathies, and B cell leukemia. Ensuring safe and effective gene transfer is crucial to the development of LV, which in turn demands meticulous examination and selection of viral genome components. Improved promoters, envelope proteins with specialized functions, and expression systems that may be inducible and regulated are the current areas of research interest. Still, problems like expensive manufacturing costs and random insertional mutagenesis are there. Because of its ability to undergo ex vivo transduction and reintroduce itself into blood flow, LV has excellent performance in the immunological and haematological systems.

Current treatment protocols of HNSCC

Surgery and (Chemo-) Radiotherapy

Rapid advancements in cancer treatment over the past 20 years have resulted in a more genetically informed approach to treating many different types of cancer, with the ultimate goal of using targeted agents in personalized strategies to lessen side effects and maximize effectiveness. Radiation therapy and surgery, with or without concurrent cisplatin-based chemotherapy, are the cornerstones of HNSCC

treatment, despite extensive research into potential novel targeted therapies. Genetics and hr HPV presence are not yet included in treatment planning; instead, it is dependent on tumor location, tumor stage, imaging, and post-operative histology results.

Responses are still underwhelming, despite the fact that treatment methods are sometimes harsh and may lead to patients' deformity and toxicities. About 30% of cancers are detected in their early stages, and for these patients, the standard of care is a single-modality approach that may include radiation or surgical removal, based on where the tumour is located. A full recovery is typical after treatment, and the 5-year survival rate is about 90%.

However, 70% of individuals arrive with advanced illness, meaning they have metastasized to lymph nodes in their area or even elsewhere. For more advanced malignancies, the treatment options include upfront surgery with post-operative (chemo) radiotherapy, concurrent chemoradiotherapy and, if necessary, surgical salvage. Some facilities provide neoadjuvant (chemo) radiation as a precondition to surgery. Since 1977, cisplatin and concurrent locoregional radiation have been the chemotherapeutic modalities of choice. Patients who are not candidates for platinum-based treatment or who have recurrent or metastatic illness are being treated with immunotherapy using anti-PD-(L)1 antibodies, cetuximab, an antibody that targets EGFR, or invasive combination chemotherapy (Figure 1).

Among the several cytotoxic drugs used in chemotherapy, cisplatin is most commonly administered in conjunction with radiation. The medication may crosslink the genomic and mitochondrial DNA strands by creating bridges between them, as well as between them. Because the replication fork has a harder time passing through this covalent interstrand crosslink, DNA replication is slowed down. In order to repair these DNA crosslinks, the FA/BRCA-pathway is essential (Figure 1). As a radiation-sensitizer, cisplatin has varying effects on different types of malignancies. Aside from a faulty FA/BRCA pathway, no biomarker or scientific explanation has been found for the response to cisplatin. Due to cisplatin-induced toxicity, many HNSCC patients end up in the hospital and are unable to complete their treatment regimen. The last point is that cisplatin is or might be ineffective against cancers.

Approximately two fractions of 70 gerson milligrams of photons (IR) are administered to the majority of HNSCC patients. Figure 1 shows that a large proportion of Reactive oxygen species (ROS) produced by water radiolysis in the presence of radiation (RT) are what cause single strand DNA (ssDNA) breakage which in turn causes DNA peroxides to develop. Because of these ruptures, replication forks and the activation of the G2/M-checkpoint are both halted. When replication stops at single-stranded DNA (ssDNA) breaks or when unrepaired ssDNA breaks transform into double-stranded DNA (dsDNA) breaks, DNA damage occurs. Common methods for repairing radiation-induced double-strand DNA damage include Non-homologous end joining (NHEJ) and microhomology-mediated end joining (MMEJ). Techniques for DNA repair NHEJ and MMEJ are prone to errors, which might introduce mutations that could be fatal. On the other hand, reports have shown that MMEJ might enhance IR-resistance.

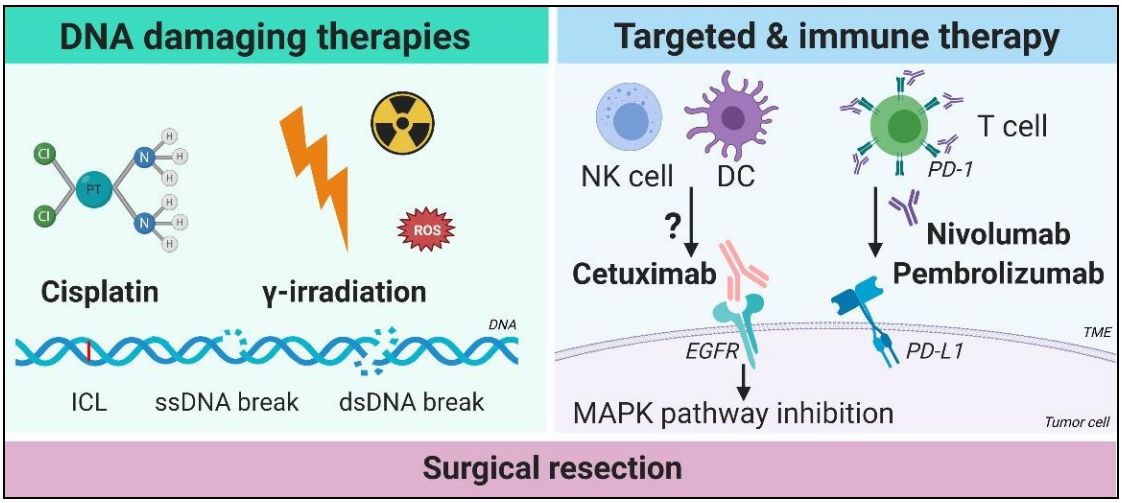

Fig 1: Approved and clinically applied treatment interventions for HNSCC

Identification of essential genes in (PRE) HNSCC cells by descriptive and functional genomics

In order to pick biomarkers for clinical classification and to preclinically identify vulnerabilities of HNSCC, a fundamental knowledge of tumor cell biology is necessary. Taken as a whole, they represent the baby steps toward more effective and tailored therapies for HNSCC. A plethora of novel molecular information has been uncovered by genomics datasets collected by groups like as the TCGA. See below for a comprehensive overview of the driver mutations and chromosomal changes that cause HNSCC, as provided by these datasets.

The TCGA failed to achieve its goal of identifying new driver genes that may be targeted by small molecule inhibitors in HNSCC since there are no oncogene drivers in this kind of cancer. Recent technological developments have enabled us to use a more functional genomics approach, while both descriptive and functional genomes have added to our knowledge of the aetiology of HNSCC. Pharmacological library screenings, genome-wide array-based small interfering RNA (siRNA) screens, microRNA expression screens, (kinwomen) short hairpin RNA

(shRNA) drop-out screens, and inhibitors that target essential genes in HNSCC cells are among the many approaches being used by researchers.

shRNA (Kinome) Drop-Out Library Screens

When bound to gene transcripts, short interfering RNAs-which are made of molecules with 20–25 nucleotides and two strands of RNA-either degrade the transcript or block translation. These small interfering RNAs (siRNAs) may be cloned and introduced into lentiviral or plasmid vectors as short hairpin RNAs (shRNAs). Libraries may target either the whole genome or a specific region of it by combining siRNAs and shRNAs. To find kinases that can be targeted, scientists conduct functional RNA interference kinome screening. After resistance-marker selection, cells are cultured for an extended period after being infected with a lentiviral library that contains the combined shRNAs. The cells are then spread out on huge culture plates. From the time cells are first infected (t0) until the completion of the research Genomic sequencing reveals an increase or reduction in the relative abundance of shRNA constructs that promote or hinder cell proliferation. Because of the possibility of off-target effects with shRNAs resulting to false-positive hits or the possibility of cells being infected with numerous shRNAs causing a particular lethality, To ensure the discovered hits are authentic and important, further validation is required. The use of small interfering RNAs (shRNAs) and partial knockdown of target genes is the most effective way to imitate medication inhibition; nevertheless, these screenings run the risk of omitting crucial genes. Notwithstanding these caveats, it is noteworthy that shRNA screens performed in HNSCC revealed the essentiality of several genes controlling the DNA damage response and cell cycle.

Drug Library Screens

To save time, many researchers only use drug libraries to scan cancer cells for weaknesses. It is possible to test for specific inhibitors that may affect cell line viability *in vitro* using one of the many commercially available pharmacological libraries. The goal of doing various the goal of conducting drug screens using arrays in 2D tissue culture of HNSCC cells and primary HNSCC cells is to identify new therapeutic targets or combinations of drugs that work synergistically. Screening for HNSCC cell survival-affecting inhibitors of the cell cycle and DNA damage response yielded many hits. The Cancer Genome Project of the Welcome Sanger Institute and the Mass General Hospital Cancer Centre is known as the Genomic Dataset on Drug Sensitivity in Cancer (GDSC) is one of many cancer cell line vulnerability databases. This includes HNSCC. Acquiring survival statistics Researching oral premalignant cells via the use of pharmacological libraries to target high-risk premalignant alterations is an attractive prospect. Moving on with clinical studies based on *these in vitro* results, further research is required. One major drawback of drug screens is their inherent bias; for example, There are a lot of inhibitors that block a lot of proteins without really targeting them, making it more difficult to comprehend how a medication works and providing less insight to the core mechanism of biology.

Gene therapy approaches for head and neck cancer

Better anticancer treatments with less treatment-related side effects should be possible if we could identify and destroy the particular genetic abnormalities that cause carcinogenesis and cancer development.

When expressed at the correct location and concentration, any one of a number of known genes may effectively target and destroy cancer cells. Although it would be ideal to administer gene therapy systemically to patients with metastatic cancer, At this time, there is no proof that this method is secure or productive. This kind of cancer is well-suited for gene therapy since it has the potential to effectively treat both primary and secondary tumours in the head and neck. In a genetic treatment approach to cancer, there are numerous broad tactics that are used, like as.

Gene addition therapy

This method introduces tumor suppressor genes that render carcinogenic cells inactive, hence regulating tumor development. The abnormal regulation of cell cycle progression by mutations and overexpression of cell cycle regulators is a hallmark of cancer cells. Some of the genes that are altered in instances of head and neck cancer include the retinoblastoma gene (Rb1), P53, P16, and P27. Two common signalling routes that maintain adult cell cycle phases-G0 and G1-are the Rb and P53 pathways, respectively. The tumour suppressor genes (P53, P16, and P27) and the protein products of several proto-oncogenes have a substantial influence on this pathway. Cancer may develop when some of these genes become mutated, leading to an imbalance in cell growth control. The Rb protein controls when cells exit G1 phase, whereas P53 determines whether cells will be programmed to undergo apoptosis or have their cell cycle halted in reaction to stress or DNA damage. Patients with squamous cell carcinoma were studied by injecting an adenoviral vector expressing wild type P53 directly into the tumor.

Cancer gene therapy using oncolytic viruses

A very encouraging method of Oncolytic viruses, which replicate only in tumour cells, are used in gene therapy. Retroviruses, adenoviruses, and herpes viruses are often used in gene therapy. Adenoviruses deficient in E1B were the first to be used as oncolytic agents; these viruses cannot replicate in healthy cells but may infect those that lack the P53 gene, a feature shared by most tumor cells. When retroviruses infect a cell, they cause the cell to undergo reverse transcription, leading to the creation of DNA with two strands. The incorporation of DNA into the host genome allows genes to be transmitted down across generations. The ex vivo transfer of drug-resistant genes is one strategy where retroviruses might be effective into leukocytes. The use of an HSV-1-TK murine retrovirus in conjunction with intravenous ganciclovir has been the subject of large-scale, international glioma studies.

Clinical evaluation of replication-defective (Rd) adenovirus vectors for somatic cell gene therapy

Due of their high immunogenicity, ads need extensive modification to halt the gene from leaking out and causing CTL to kill transduced cells in the body. To add insult to

injury, many people already have antibodies to Ad, particularly the serotype most often employed in gene therapy vectors (Ad5). When an individual already has antibodies, they won't be able to "take" the Ad vector. The field made great strides forward thanks to clinical trials using RD Ad vectors, even though it became apparent that these vectors might not be the best option for long-term gene complementation in monogenic diseases. This is because a wealth of data was gathered during the early trials about the safety of these vectors.

Two papers published by Ronald Crystal and colleagues reviewed the literature on the topic of local delivery of RD Ads expressing CFTR, VEFG, or E. coli cytosine deaminase. The investigations included a variety of trials. The intranasal drops or intrabronchial catheter were supplied to 90 individuals in the experiments, intramyocardial or skeletal muscle injection, or direct intratumoral injection, respectively. With the trial cohort as a whole, 319 adverse events were recorded; however, out of these, only one was determined to be Ad-related. The other 218 were attributed to individuals' underlying medical conditions. Samples of blood, urine, and swabs taken from the nose, rectal, and pharyngeal cavities were tested to determine if the vector is still present one and seven days after injection in order to identify any potential shedding. We identified no RC virus (which may have arisen during in one of the 1,685 samples (a recombination event) we tested, and one of those samples shed its initial RD vector. Similar to the previous trial, patients in this one had mild clinical symptoms as fever, myalgia, and arthralgia after receiving CFTR from an RD Ad vector either bronchoscopy or aerosol therapy.

Conclusion

This study demonstrates that early clinical studies of cancer gene therapy have shown promising results. The more we learn about the genetic basis of cancer, the more we can use these concepts to specifically attack tumor cells. It may soon provide a final solution for head and neck malignancies, significantly lowering the high death rate linked with these lesions while offering superior efficacy compared to existing treatments. Clinical studies for gene therapy are progressing from Phase 1 and 2 to Phase 3 and 4, however proving the medicines' actual effectiveness will take a long time and include many patients. When combined with pre-existing therapeutic regimens like radiation, chemotherapy, and surgery, gene therapy is expected to have a significant impact. Using gene therapy in conjunction with immunotherapy, chemotherapy, and radio treatments has the potential to kill more cells with less side effects, according to a growing body of research. As a promising new area of cancer treatment, cancer gene therapy is quickly coming of age.

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