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Role of gene therapy in treatment of cancer for craniofacial regeneration—current molecular strategies, future perspectives, and challenges: a narrative review

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Gene therapy involves the introduction of foreign genetic material into host tissue to alter the expression of genetic products. Gene therapy represents an opportunity to alter the course of various diseases. Hence, genetic products utilizing safe and reliable vectors with improved biotechnology will play a critical role in the treatment of various diseases in the future. This review summarizes various important vectors for gene therapy along with modern techniques for potential craniofacial regeneration using gene therapy. This review also explains current molecular approaches for the management and treatment of cancer using gene therapy. The existing literature was searched to find studies related to gene therapy and its role in craniofacial regeneration and cancer treatment. Various databases such as PubMed, Science Direct, Scopus, Web of Science, and Google Scholar were searched for English language articles using the keywords "gene therapy," "gene therapy in present scenario," "gene therapy in cancer," "gene therapy and vector," "gene therapy in diseases," and "gene therapy and molecular strategies."

Keywords: Craniofacial regeneration; Genetic therapy; Neoplasm; Vector

Introduction

The human genome consists of approximately 25,000 genes that encode an expanded category of proteins commonly known as the building blocks of the cell that initiate each biological process [1,2]. Some genes remain unchanged but show changes due to disruptions, mutations, and deletions. These basic and uncontrollable genetic modifications transform protein functions to directly influence the cell structure and physiological appearance of severe diseases, disorders, and deficiencies [3,4]. Gene therapy is a unique method for treating various diseases by transferring a recombinant gene. Gene therapy is a favorable therapeutic option for various diseases such as viral infections, hereditary disorders, and cancer. Various gene delivery techniques have been reported for gene therapy [5,6].

Currently, gene therapy is being investigated only for diseases for which there is no alternative therapy. Genetic molecules must enter the nucleus of host cells to activate gene expression. Gene therapy transmits genetic instructions to somatic cells for the production of distinct therapeutic proteins that regulate genetic diseases.

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It is important to develop an interaction between the gene delivery system and target cells to create a competent gene delivery system [7,8]. Alipogene tiparvovec (Glybera; UniQure, Amsterdam, the Netherlands), the earliest gene therapy product, was certified by the European Medicines Agency. This was considered a notable initial advancement in gene-based medicine [9,10].

Methods in gene therapy

These methods include gene modification, gene transfer, gene transfer to specific cell types, and gene insertion. Gene modification is a replacement treatment and modified gene therapy is a method that corrects flawed genes. In substitute therapy, a natural gene is replaced with a nonnatural gene via recombination. Gene transfer methods utilize chemical, biological, and physical means for gene transfer. Gene transfer to specific cell types can be divided into gamete gene therapy and somatic gene therapy. Gene insertion (eugenic approach) or gene injection is another gene therapy method. Other modes involving genetic engineering include gene targeting and the eradication of specific genes through nuclease engineering, such as developing I-CreI homing endonucleases, zinc-finger nucleases (ZFNs), or transcription activator-like effector nucleases (TALENs). These methods are currently used in clinical trials [11].

Gene therapy history and future

On September 14, 1990, a girl was treated by Dr. William French Anderson and colleagues at the National Institutes of Health Clinical Center in Bethesda, MD, USA. White blood cells were extracted from the patient. After gene implantation, the cells were transferred back into the patient's body. Noticeable progression of the immune system was observed. Currently, gene therapy trials for various diseases are ongoing. Patients with melanoma and other skin cancers have been treated using gene therapy techniques [12].

Technologies for gene delivery

From the time of development of recombinant DNA methods, safe, and efficacious administration of gene products has been a major challenge. Gene delivery is performed using a vector. A vector adequately delivers a gene to a target tissue, allows the delivery of a gene of sufficient size, and accomplishes correct transgenic expression to regulate the defective gene. The distribution of gene products is achieved using viral vectors, nonviral vectors, and bactofection [13].

Ideal properties of vectors

The properties of ideal vectors include the following: (1) the vector should be stable and easy to produce; (2) it should possess low toxicity, high efficiency, and high specificity; (3) it should be nonimmunologic and inexpensive; and (4) ideally, it should be able to deliver DNA into the cell nucleus [13-15].

Viral vectors

Currently, viral vectors are the most favored vectors. Viruses deliver their genes to human cells in a pathogenic manner. Scientists favor this efficiency, replacing parts of the viral genome with the gene therapy candidate. This recombinant virus may be utilized to transfer genes inside cells. Various categories of viruses can be used as gene therapy vectors. These include adenoviruses, retroviruses, and adeno-associated viruses [16].

1. Adenovirus

Adenoviral vectors are non-enveloped, double-stranded (ds)-DNA viral vectors. Adenoviral vectors are most extensively used for gene delivery. Adenoviral DNA does not integrate into the genome or replicate during cell division. This limits the use of adenoviruses to basic investigations, although adenoviral vectors have been used in both *in vivo* and *in vitro* studies. The primary uses of this vector are in vaccination and gene treatment [17,18].

Considering that humans are frequently exposed to wild-type adenoviruses, the majority of individuals have established neutralizing antibodies that intercept the virus before it arrives at its target cell. Adenovirus-mediated gene therapy has been used to treat chronic pain in a rodent model. These studies have encouraged the development of criteria for chronic pain therapy of the central nervous system. Adenovirus-mediated gene therapy has also been used to treat liver cancer. Gendicine (Shenzhen SiBiono GeneTech, Shenzhen, China), a p53-based adenoviral vector, was the first gene therapy certified for the treatment of head and neck cancer. Compared to retroviruses, adenoviruses infect a larger array of cells [19,20].

2. Retrovirus

Retroviruses were the first viruses to be used in gene therapy experiments as vectors. They are considered the backbone of the existing concept of gene therapy. Recombinant retroviruses can integrate into the host genome in a balanced manner. They encode a reverse transcriptase that produces DNA copies of the RNA genome. They also encode an integrase that permits integration into the host genome. Retroviruses have been utilized in various U.S.

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Food and Drug Administration (FDA)-recognized clinical studies such as the SCID-X1 study [21,22].

3. Adeno-associated virus

Adeno-associated viruses are extremely promising vectors. They can infect a wide variety of cells. They are small-scale viruses that belong to the parvovirus classification and have single-stranded DNA. They may integrate their genome at a definitive location on chromosome 19. Some scholars have presumed that many individuals harbor adeno-associated viruses that do not elicit an immune response or cause disease. Scientists have conducted animal experiments using adeno-associated viruses to amend genetic deformities. The principal shortcoming of adeno-associated viruses is that they are tiny and exclusively encode only two genes in their wild-type form. Hence, their applicability is restricted. As the virus integrates its genes directly into the DNA of the host cells, it causes unpredictable damage. Researchers face difficulties in producing large quantities of recombinant viruses. Recently, this problem was resolved by Amsterdam Molecular Therapeutics [23].

4. Lentivirus

These viruses are a retrovirus subtype. They are periodically used as vectors for gene therapy. A unique feature of lentiviruses is their ability to integrate into the genome of nondividing cells [24]. When the virus invades cells, the viral RNA genome is reverse-transcribed. It is then integrated at a random position in the genome by an integrase enzyme. The vector, now known as a provirus, persists in the genome and is passed on to the cell's progeny [25].

5. Vaccinia virus

Vaccinia virus is a complex and enveloped virus belonging to the *Poxviridae* family. It contains a dsDNA genome encoding approximately 250 genes [26]. Vaccinia virus produces four infectious forms during its replication cycle. These are the extracellular, intracellular, cell-associated enveloped, and intracellular mature virions [27,28].

6. Herpes simplex viruses

They are members of the *Herpesviridae* family and cause viral infections in most humans [29].

7. Electroporation

This is the transitory permeabilization of cell membranes of the target tissue by application of an electric field, which results in the penetration of DNA molecules into the nucleoplasm and cytoplasm of the cell [30,31]. These DNA molecules are initially present in the medium surrounding the membranes. Electroporation is mainly utilized *in vivo* for different tissues such as muscle, skin, and lung [32-34].

8. Hydrodynamic delivery

Hydrodynamic delivery is a highly efficient and simple technique for rapid intracellular distribution of water-soluble particles and compounds within internal organs [35,36]. The hydrodynamic method has been successfully used for the delivery of genes into rodent liver for the expression of erythropoietin, cytokines, and hepatic growth factors [37-39].

9. Chemical methods

Chemical delivery systems include oligonucleotides, lipoplexes and polyplexes, dendrimers, and inorganic nanoparticles such as gold, silica, and iron oxide [40].

Present molecular approaches in cancer gene therapy

Gene transportation technology permits an ample spectrum of therapeutic potential that can be integrated with traditional therapies or used to implement new treatment approaches. New delivery systems for cancer treatment and eradication are currently being studied. As a result, the use of various nucleic acid-based systems such as TALENs, interfering RNA (iRNA), ZFNs, recombinant DNA, suicide genes, and clustered regularly interspaced short palindromic repeats have provoked much interest within the scientific community [41-43].

1. Antisense oligonucleotide technology in cancer

Antisense oligonucleotides are characterized as highly modified synthetic DNA or RNA oligonucleotides that are designed to selectively target gene-encoded RNA molecules through Watson-Crick base pairing. The binding of antisense oligonucleotides to their corresponding targets can generate unique mechanisms of action [44,45]. These mechanisms can be categorized as those that promote RNA degradation and those that confine RNA and hinder its activity without activating RNA degradation. Regardless of mechanism, antisense oligonucleotide-mediated intervention is a promising therapeutic approach for cancer treatment [46,47].

2. Interfering RNAs

RNA interference was first described in *Caenorhabditis elegans* in 1988. This technology has made appreciable advances in cancer treatment. iRNA is a dsRNA-mediated gene silencing method. This mechanism recognizes and targets pathogenic dsRNA parti-

cles for destruction. Three types of small RNAs have been identified in animals, specifically small interfering RNAs (siRNAs), microRNAs (miRNAs), and piwi-interacting RNAs (piRNAs) [48-50].

Some miRNAs are overexpressed in cancer and induce tumor development, whereas others are downregulated and block inhibitory control over a few oncogenes [51,52]. siRNA is eminently selective toward its target microRNA. Compared with miRNAs, this selectivity is greater, as siRNAs can distinguish between sequences with even a single distinctive nucleotide [53]. piRNAs are mainly involved in epigenetic regulation [54]. Short hairpin RNAs (shRNAs) are also used for RNA interference. These shRNA molecules are synthesized from expression vectors inside the nucleus of the cell, transferred to the cytoplasm, and processed by automatic machinery to produce shRNAs [55-57].

Gene therapy for craniofacial regeneration

The regeneration or reconstruction of oral tissue, in addition to craniofacial tissue, is a difficult process that requires a blend of engineering technology and clinical and basic science. The recognition of the relevant stage, spatial and temporal signals, and cell sources is essential for enhancing the development of single tissues, tissue interfaces, and hybrid organs consisting of numerous tissues. The regeneration of craniofacial tissues using gene therapy utilizes genetic vectors as supporting building blocks for tissue repair and growth. The harmonious association between craniofacial tissue engineering and viral gene therapy substantially strengthens the potential for regenerating and repairing tissues *in vivo*.

1. Head and neck squamous cell carcinoma

The treatment of head and neck squamous cell carcinoma (HNSCC) is the most advanced application of gene therapy in the craniofacial region. Three main strategies are used to target each solid tumor using genetic treatments.

First, immunomodulatory treatment enhances the perceptibility of tumor cells to the immune system *in vivo* or customizes effector cells to enhance their tumor-targeting capacity by establishing the expression of a particular gene [58]. Second, oncolytic viruses have evolved to select a target cancer cell, enter, and destroy it. Third, suicide genes can be imported into cancer cells to broaden cell sensitivity to antiviral drugs like acyclovir [59-61]. Additional strategies for targeting HNSCC include the introduction of endostatin, genes encoding p53, and antiviral interleukin (IL)-2 or IL-12 [62-65].

2. Mineralized tissues

Gene therapy based on animal models and the architecture of a particular craniofacial structure, such as cartilage or bone, has provided an advantageous and innovative way to regenerate composite mineralized tissues, such as the temporomandibular joint and tooth [66,67]. Delivery of bone morphogenetic proteins and platelet-derived growth factors (e.g., PDGF-B) at the location of the periodontal deformity is known to increase the healing and repair of gingiva and bone. At the therapeutic level, gene delivery permits the sustained synthesis of proteins in defined locations [68-70].

3. Salivary gland

Insufficient function of the salivary gland can occur due to the side effects of radiation therapeutics or as an aftereffect of autoimmune diseases, such as Sjögren syndrome. Researchers are engaged in designing salivary gland alterations that are perhaps confined to the location of the parotid gland. Acinar cells in the salivary gland require membrane proteins to develop an osmotic gradient for the unidirectional movement of fluids [71,72].

Salivation occurs because of the reaction to agonists that increase intracellular Ca^{2+} concentrations and is promoted by an osmotic gradient that directs the movement of fluid by means of water passage through apical membrane proteins called aquaporins (AQPs). It has been observed that confined ductal epithelial cells do not express AQPs, and hence are unable to mediate fluid movement [73,74].

4. Wound healing of mucosa

For esthetic reconstruction in persons mutilated by surgery, trauma, or severe burns, a proportionate structure of mucosa and skin is necessary. For coverage of wounds and burns, Apligraf (Integra LifeSciences, Princeton, NJ, USA) and Dermagraft (Organogenesis Inc., Canton, MA, USA) are used [75,76].

Gene remedies for cancer treatment

Cancer develops because of the loss of regulation of normal cell apoptosis and proliferation. Advancements in cancer treatment require unique remedies with unusual mechanisms of action, different means of cell death, and harmony with traditional therapies. Gene therapy has all these characteristics. Various gene therapy approaches have been established for cancer treatment. These approaches include suicide gene therapy, oncolytic virotherapy, antiangiogenic gene therapy, immunotherapy, gene-directed enzyme prodrug therapy, and siRNA therapy. Immunogenetherapy is a promising treatment method for p53-deficient tumors (e.g., Yescarta [axicabtagene ciloleucel; Kite Pharma, Inc., Santa Monica,

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CA, USA], Imlygic [talimogene laherparepvec; Amgen, Inc., Thousand Oaks, CA, USA], Kymriah [tisagenlecleucel; Novartis International AG, Basel, Switzerland], and Gendicine) [77].

1. Oncolytic virotherapy

This is the most promising method of tumor immunotherapy. Oncolytic virotherapy utilizes replication-competent viruses that grow rapidly, particularly in tumor cells. Genetically modified or commonly occurring oncolytic viruses are used. Viruses such as measles, vaccinia, and vesicular stomatitis viruses are genetically modified to decrease viral pathogenicity and enhance tumor specificity. Wild-type viruses, such as the Newcastle disease virus or parvoviruses, are carefully replicated in tumor cells without gene modification. Oncolytic viruses respond by precisely lysing tumor cells and inserting wild-type tumor suppressor genes into cells that do not express these genes [78,79].

2. Gendicine (recombinant human p53 adenovirus)

Gendicine is a nonreplicating adenoviral vector that was first accepted as a gene therapy for HNSCC in 2003. The E1 gene in the adenoviral genome was replaced with the tumor suppressor p53 complementary DNA (cDNA). In tumor cells, the expression of wild-type p53 has an antitumor effect by triggering the apoptotic pathway and relieving blockage of DNA repair and apoptotic p53 translocation. p53 gene mutations are prevalent in various cancers. Hence, Gendicine promotes the function of p53, restores its translocation, and damages tumor cells [80,81].

3. Oncorine (rAd5-H101)

Oncorine (Shanghai Sunway Biotech, Shanghai, China) is an oncolytic recombinant adenovirus-5 (with a deletion in the *E1B55K* gene) recognized for the treatment of refractory nasopharyngeal cancer. Elimination of the *E1B55K* gene results in the inhibition of viral expansion in normal cells, permitting proliferation only in p53-impaired host cells. After the lysis of cancer cells, the adenovirus is released and infects other cells, leading to Oncorine-mediated cell death [82,83].

4. Imlygic

Imlygic is an inherently altered oncolytic type 1 herpes simplex virus (HSV-1) used for the treatment of metastatic melanoma. The HSV-1 genome was modified by substitution of the α47 and γ34.5 genes with agranulocyte-macrophage colony-stimulating factor cDNA. The deletion of γ34.5 results in the suppression of pathogenicity and tumor cell-selective replication. During viral infection, the γ34.5 gene normally hinders the translation of host-cell proteins in favor of viral production. Hence, suppression of γ34.5 blocks proliferation of the virus in normal cells [78,79].

5. Rexin-G

Rexin-G (Epeius Biotechnologies Corp., San Mateo, CA, USA) is a major targeted injectable vector accepted for the treatment of various cancers. It has a signature (SIG)-binding peptide that binds to abnormal SIG proteins in tumor cells, which results in an increase in vector accumulation in tumor cells, and it expresses human cyclin G1 inhibitor. After entry into tumor cells, it expresses the cytocidal fibronectin 1 protein, which results in inhibition of the cell cycle, leading to apoptosis [84,85].

6. Chimeric antigen receptor T-cell therapy

This therapy uses T cells that have been altered *in vitro* to express chimeric antigen receptors (CARs) that specifically recognize tumor-associated antigens. CAR is known as "chimeric" because it consists of the antigen-binding domain of the B-cell receptor and the T-cell receptor activation domain. Stimulated CAR T cells provide target-distinct memory cells that promote tumor regression [86].

7. Kymriah (tisagenlecleucel)

Kymriah is used to treat relapsed B-cell acute lymphoblastic leukemia and was the first FDA-permitted CAR T cell-positioned gene therapy. Kymriah consists of T cells, which are mutated with lentivirus to encode a CAR consisting of murine single-chain variable fragment, which is specific for CD3 zeta, the CD8 transmembrane hinge, intracellular domain 4-1BB (CD137), and CD19. Kymriah, after binding to CD19, commences its antitumor activity via the CD2 domain [87,88].

8. Yescarta (axicabtagene ciloleucel)

Yescarta is used to treat aggressive non-Hodgkin lymphoma. It is based on CAR T-cell therapy. Yescarta encodes a CAR that consists of extracellular murine anti-CD19, which binds to the cytoplasmic region carrying the CD3 zeta and CD28 co-stimulatory domains [89,90].

9. Zalmoxis

Zalmoxis (MolMed S.p.A., Milan, Italy) is used to treat various hematopoietic malignancies. Zalmoxis consists of genetically altered allogeneic T cells, which have been transduced to express a truncated human low-affinity nerve growth factor receptor and HSV-1 virus thymidine kinase.

The administration of genetically altered donor T cells rebuilds immunity to resist infection. However, the donor cells may attack

host cells in graft-versus-host disease. Zalmoxis provides immune reconstitution, graft-versus-leukemia improvement, and post-transplant graft-versus-host disease control [91,92].

Risks affiliated with viral vectors

The most common concerns associated with viral vectors are the risk of inflammation, insertional mutagenesis, and off-target effects [93,94]. Inflammation was observed in the death of Jesse Gelsinger in 1999 resulting from an excessive dosage of adenovirus. Insertional mutagenesis is a major challenge that must be overcome when using gene therapeutics. Sometimes, the vectors integrate into undesirable regions of the genome. To avoid this problem, using a vector that does not integrate is advisable [95].

Challenges and the way forward

Gene therapy has been widely used since its discovery as a treatment option for cancers, neuronal and infectious diseases, and metabolic disorders. Gene therapy is effective in treating Leber congenital amaurosis, beta-thalassemia, and immunodeficiency diseases such as adenosine deaminase severe combined immunodeficiency. For the large-scale production of viral particles, different approaches, such as insect cell-based baculovirus expression and cell lines expressing capsid proteins, are useful [96].

Conclusion

Various gene therapy trials are underway for single-gene and complex diseases, and vector collection and engineering approaches have been greatly enhanced, as apparent by the number of recent stage III studies. Although the gene therapy field has been accustomed to major obstacles and limited success, it is particularly important to encourage active areas of medical research. Interest in this therapy has been established based on its potential to treat and cure some of the most virulent and overwhelming diseases affecting individuals. The goal of gene therapy is to express therapeutic genes in host cells to produce favorable biological effects. However, the effectiveness of current strategies is inadequate for realizing the full potential of gene therapy.

Gene therapy has the capability to improve genetics by correcting an altered gene or performing site-specific modifications intended for therapeutic treatment. This treatment has been achieved through advances in genetics and biotechnology that allow the manipulation of vectors to deliver extra chromosomal agents to target cells. New experimental vector designs, improved efficiencies, specific delivery systems, and a better understanding of the induction of inflammatory responses can improve safety and extend the technology for clinical utilization.

Thousands of clinical studies have been conducted since the introduction of gene therapeutics in humans. It is supportive that number of gene therapy trials for various disorders are now completed. The selection of vectors and their design strategies have substantially improved. In the future, gene therapy will surely help in the medical field, with promising results in the management of diseases with limited treatment options.

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