Gene Therapy For Neurological Disorders: Parkinson's Disease And Glioblastoma

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ABSTRACT

Gene therapy, though first proposed as a concept decades ago, has faced numerous setbacks in the form of serious adverse effects during early clinical trials. However, recent successes in the field as applied to neuromuscular disease, blindness, and cancer have stimulated renewed interest in the application of gene therapy for clinically intractable diseases. Neurological diseases, which have been proven to be some of the most lethal and hardest-to-treat disorders, lack and require additional treatments and attention. In this review, we focus on the methods of gene therapy, and how this type of treatment, delivered through a diverse repertoire of vector systems, is showing promising results in treating patients suffering from glioblastoma and Parkinson's disease.

Introduction

Neurological disorders are currently affecting around 1 billion people globally (Bulck et al., 2019). They take 2 of the top 3 spots on the leading cause of death in high-income countries in 2019 (WHO, 2020), and are currently considered as the leading cause of disabilities in the world (Dorsey et al., 2018). As one of the most common forms of health issues, neurological disorders cover a wide range of diseases from simple headaches to life-threatening brain tumors (Bulck et al., 2019). Alzheimer's disease, for example, afflicted 46.8 million patients worldwide in 2015. Parkinson's disease, in addition, affected another 6.1 million patients in the same year (Dorsey et al., 2018) and is projected to reach around 17 million patients by the year 2040 (Dorsey et al., 2018). Current therapies for neurological disorders range from surgeries to medications. However, often these therapies fall short in curative capacity. For example, current FDA-approved treatments for glioblastoma, the most common but malignant form of brain tumor, are surgical excision, chemotherapy, and radiation therapy but they only result in a 6.8% 5-year survival rate (Davis, 2016; Wen et al., 2020). Recent advances in gene therapy have the potential to directly address the root causes of neurological disorders. Gene therapy is the introduction, by viral and non-viral methods, of genetic material into the cells of the patient. Although it has seen some success in other types of diseases, gene therapy faces the additional challenge of overcoming the blood-brain barrier when applied to neurological diseases. This barrier, composed of brain cells that act as the security guards of the brain, allows only a certain kind of substance to enter the brain (Sudhakar & Richardson, 2019). This review will examine the current clinical advances in gene therapies for neurological disorders, possible advancements that could be made to enhance the quality of gene therapies, and ways that existing challenges faced in the neuro-medical field could be conquered by gene therapies. We will specifically focus on brain cancer and Parkinson's disease, which are two highly prevalent yet clinically intractable diseases.

Gene Therapy

What is it?

Gene therapy is the manipulation of genetic material to correct deficits in protein production or delete the production of aberrant proteins. In simpler terms, it is the delivery of therapeutic genes that correct genetic abnormalities in the human body (Choong et al., 2016). The process of gene therapy is the following. First, the disease being treated and the mutated genetic sequence responsible for the disease is identified. Next, the multiplication of the therapeutic gene that will be used is performed. A common method for this step is the use of PCR. When the cloning is done, the cloned genes are transported to the designated area in the patient's body through vehicles called vectors. Once they reach the destination, the vectors will unload these genetic materials, eventually creating the desired protein products (Smith & Blomberg, 2017).

What are the methods?

Currently, there are three main methods of gene therapy, which are all similar in all procedures besides the use of different ways of transportations. They are composed of the use of viral vectors, non-viral vectors, and engineering vectors for gene therapy (Ramamoorth and Narvekar, 2015). In this review we will focus on viral vectors, Non-viral and engineered vectors are comprehensively reviewed elsewhere (Ramamoorth & Narvekar, 2015; Li & Samulski, 2020). Viral vector gene therapy is the use of modified viruses as means of transportation for therapeutic genetic materials. This type of gene therapy has a natural advantage due to viruses' ability to deliver genes into target cells accurately and efficiently. Viruses used in this type of gene therapy are modified in a way that prevents them from replicating harmfully (Chen et al., 2018). Though all viral-based gene therapy uses viruses as vectors, there are different kinds of viruses that can be chosen. AAV, or Adeno-Associated Virus, is one of the most popular and safest of all due to its unique characteristic of the lack of an envelope, which makes it possible to generate AAV units with the complete absence of viral genes but the ability to carry interested genes (Naso et al., 2017). Lentiviral vectors, or LV, is another example of a secure and fast way to move a therapeutic gene to a target cell. It is a derivative of the human immunodeficiency virus and thus is mostly used to generate immunity or fix immunodeficiency when immune cells such as T-cells are set as the destination (Milone & O'Doherty, 2018). Besides AAV and LV, there are other groups of viruses that can act as vectors, however, this review will only focus on the two listed above.

Past success

Gene therapy has had numerous successes over the past two decades, the most notable being the development of an AAV-based drug for gene therapy in the eyes. The foundational research for this drug was laid in 2008. Three independent study groups all demonstrated the safety and effectiveness of a subretinal injection of retinal pigment epithelium 65 (RPE65), a 65kDa protein, through AAV vectors. This specific protein helps with the improvements of vision in patients who suffer from inherited blindness. 9 years later, the same technology was developed as a drug called LUXTURNA™ (voretigene neparvovec-rzyl) by Spark Therapeutics, Inc. (Philadelphia, PA). Making it the first FDA-approved gene therapy drug for the eyes (Rodrigues et al., 2019).

Overcoming the blood-brain barrier

The blood-brain barrier (BBB) is an important layer of defense laying between the brain and the vascular system. Due to the high energy consumption rate of the brain, the vascular system delivers supplies of metabolic nutrients (oxygen and glucose) to the brain almost incessantly (Wong et al., 2013). While the necessary supplies arrive in the brain, the BBB prevents most other substances from entering our neurological systems, thus avoiding any possible danger. As a permeable layer, BBB blocks 100% of the large molecules and 98% of the small molecules attempting to reach the brain (Pardrige, 2008). To overcome the BBB during gene therapy, scientists have found ways to either penetrate or go around the barrier. The developments and modifications on AAV vectors made in the past decade enabled it to

breach the BBB easily, which is one of the reasons that makes it stand out among all the vectors. In addition, the injection of vectors in alternative sites permits the trespassing of the BBB. These sites include subarachnoid spaces, intrathecal space, and the ventricular system of the brain. Materials infused in these sites can be further distributed to target tissues via CSF (cerebrospinal fluid) and the spread of central nervous system (CNS) distribution. Lastly, recent research demonstrated certain vectors' ability to engineer ependymal and choroid cells to directly secrete the necessary therapeutic protein to the target area, which avoids the encountering of the BBB (Choudhury et al., 2018).

Methods

This literature review focused on the principles of gene therapy and neurological diseases, as well as clinical trials focusing on the application of gene therapy for the treatment of Parkinson's disease and glioblastoma. As such, peer-reviewed articles in the PubMed and National Library of Medicine databases were examined in this literature review with the following keywords: "gene therapy", "genome/gene editing", "neurological disorders", "brain diseases", "glioblastoma", and "Parkinson's disease." We used the following qualification to choose the proper research articles: papers used had to be relatively up to date considering that this is a new field in biomedical technology.

Parkinson's Disease

Application of gene therapy

There are two ways, during which both use AAV vectors, that gene therapy could be applied to Parkinson's disease. These two applications aim to use therapies to decrease the severity of the symptoms. The first approach is the genetic transfer of glutamic acid decarboxylase (GAD), which triggers the production of γ-aminobutyric acid (GABA) with glutamate. The second approach is the transfer of an enzyme called aromatic l-amino acid decarboxylase (AADC) that increases the rate of dopamine production through the conversion of levodopa to dopamine (Sudhakar & Richardson, 2019).

AAV2-GAD

This method was designed to correct the overactivity of pathogens and to increasingly inhibit GABA production in the basal ganglia node area by delivering GAD to the subthalamic nucleus (STN). Though previous observation suggests that the injection of an agonist for GABA receptors into STN effectively reduce symptoms of the disease (Levy et al., 2001), no significant improvements were observed when AAV2-GAD is transferred to the STN of nonhuman primates with MPTP, a drug used to induce Parkinson's symptoms by damaging dopaminergic neurons in the brain (Emborg et al., 2007). This method has gone through the first two phases of the clinical trial, during which the AAV2-GAD was transferred to STN of PD patients via Convection-enhanced delivery (CED). During phase I, 50 μL of AAV2-GAD was delivered unilaterally to the STN of each of the 12 patients in the following three distinct dosing groups: 1×10^{12} vg/mL, 3×10^{11} vg/mL, and 1×10^{11} vg/mL. In their Unified Parkinson's Disease Rating Scale (UPDRS), all patients displayed noticeable improvements in their symptoms up to 12 months post-injection. To further prove the efficacy of the delivery, FDG-PET scans also captured a decrease of activity in the STN. During phase II, 1×10^{12} vg/mL of AAV2-GAD was delivered bilaterally to each of the 16 subjects. Similarly, light improvements in their PD symptoms were found 6 months after the injection (Kaplitt et al., 2007; Lewitt et al., 2011).

AAV2-AADC

As a pro-drug gene therapy method, AADC injection enables a significant, constant, increase of levodopa conversion to dopamine level. The therapeutic target for this approach is the post-commissural (sensorimotor territory) striatum due to its unique ability of being unable to be deteriorated as PD progresses (Bankiewicz et al., 2006; Hadaczek et al., 2010). Pre-clinical studies prior to the trials demonstrated that the infusion of AADC is effective in MPTP-lesioned primates both immediately and long-termly (Bankiezics et al., 2000). The therapy resulted in better performance scores, fewer side effects, decreased levodopa requirements, as well as a continuous, over-expression of AADC up to 9 years post-infusion. Phase I clinical trial for this approach was done on 10 patients, each receiving 200 μL (100 μL for each hemisphere) of vectors. Half of the subjects were differentiated from others via two distinct doses (3×10^{11} vg and 9×10^{10} vg). Post-therapy FMT-PET scans (6 months post-operation) suggest that the lowest of 25% and up to 65% of the increase in AADC uptake level was observed in the subjects, in which the group receiving the smaller dose corresponds to a less significant improvement (Eberling et al., 2008). PET signals continue the demonstration of elevated signals (representing improvements in symptoms) until 4 months post-delivery. However, UPDRS illustrated a decrease in performance score only 1 year after the operation, leaving the possibility for another trial with bigger doses, which might alter the outcome (Christine et al., 2009. A second trial for phase I was put underway soon after the first. This trial significantly increased the injection volume (up to 8.8×10^{12} vectors) per patient. The result of this trial demonstrated no significant increase in levodopa uptake and UPDRS when compared to the first trial with a smaller dose. However, it did have an increased dopamine receptor binding frequency ([11C]raclopride binding) than the first trial. In conclusion, there is no significant dose-dependent effect under this approach (Mittermeyer et al., 2012).

Glioblastoma

Glioblastoma is considered to be one of the most aggressive forms of brain tumor, with a 5-year survival rate of less than 10% (Carlsson et al., 2014). Current advanced treatments such as surgical removal, chemotherapy, and radiation are often ineffective against glioblastoma. Frequent genetic alterations in glioblastoma include mutations in p53 and retinoblastoma and loss of chromosome arm 10q (Sakthikumar et al., 2020). Gene therapy to correct for these mutations is, therefore, a possible method to target glioblastoma. While deliveries through both viral and non-viral vector means are undergoing research and clinical trials, viral vector delivery was the very first approach of gene therapy for glioblastoma. Its studies started as far back as 29 years ago, making it, to some extent, a developed and mature form of treatment (Oshiro et al., 1995). In order to be effective on glial cells and neurons in the brain, researchers commonly use adenovirus and retrovirus as vectors. In recent, preclinical cases, adeno-associated viruses have shown promising results as well.

Adenovirus

One phase 1 clinical trial used adenovirus to carry Ad-p53 gene, a wildtype p53 strand, to the patient's glioma. Subjects of this trial underwent a total of two stages. During stage 1, Ad-p53 gene was injected stereotactically into the glioma using a previously implanted catheter. During stage two, the tumor-catheter complex was resected. The cavity post-resection was treated with Ad-p53. A total of 15 patients participated in the trial. Of that, 12 were treated with both stages of procedures. Exogenous p53 proteins were discovered in tumor cells' nuclei of patients. These proteins induced apoptosis of tumor cells through activating p21CIP/WAF but were only able to cover 5mm around the injection site. There were minimal side-effects and toxicity in subjects and the maximum allowed dose of injection was not reached, meaning future trials could attempt infusion of bigger doses for better outcomes (Lang et al., 2003). In another study, ganciclovir (through drugs) and intratumoral (through injection into the glioma) deliveries of HSV-tk through either adeno or retrovirus were tested and compared. The result of the trial demonstrated a pause of tumor progression in 3 of the 7 patients who underwent adenovirus vector delivery. However, all 7 patients treated with retrovirus vector

delivery experienced continuous tumor progression. Furthermore, patients' average survival rate doubled in the group treated with adenovirus than that of the group treated with retrovirus (15 months compared to 7.4 months). A conclusion was formed that the retrovirus approach experienced a failure in retrieving better outcomes due to its low brain-barrier penetration and transfection rate (Chiocca et al., 2011). Other clinical trials combined an adenovirus delivery of HSV-tk (AdV-tk) with an antiherpetic prodrug (which has the main function of inhibiting viral DNA synthesis) called valacyclovir. After injection, such prodrugs can undergo a conversion to analogs that bring toxicity and cell death to tumor cells. The conversion may also allow it to induce the activation of specific immune cells that are antitumors. Further trials in phase one used similar approaches but followed the valacyclovir injection with chemo or radiotherapy. The result was a significant increase in two and three-year survival rates of up to 33% percent. In addition, a follow-up trial presented a 4-month increase in survival time on patients treated by HSV-tk delivery (Wheeler et al., 2016) AdV-tk also showed impressive results in the treatment of pediatric glioblastoma, displaying a 16-month survival time on these patients (Kieran et al., 2016).

Retrovirus

The first trial studying the retrovirus vector approach started back in 1992. The goal of the study was to assess the combined treatment of retroviral delivery of HSV-tk with ganciclovir (Cytovene, an antiviral drug) in murine cells (Oldfield et al., 1995). In this specific trial, HSV-tk converts prodrug cytovene into its active form called ganciclovir-triphosphate after the injection. The activated drug has the ability to terminate DNA replication and thus cell division on any cancerous cell that HSV-tk has contact with (Rainov, 2000). In a different study, a trial undertook the identical approach to demonstrate this specific therapy's limitation. The result illustrated a recognizable effect on smaller tumors, but a lack of effect in subjects with a bigger, late-stage glioma (Ram et al., 1997). Moreover, the use of a different retroviral vector called Toca 511 was studied. Toca 511 was used to deliver cytosine deaminase (a suicide gene, like HSV-tk, that instigates apoptosis) alongside an oral prodrug called Toca FC. Similar to HSV-tk, CD enzymes convert Toca FC to its activated form called 5-fluorouracil (Huang et al., 2013; Takahasi et al., 2014). Under a phase 1 clinical trial done on subjects with developed glioblastoma, this approach successfully brought upon the mediation and toleration of regression of gliomas (Aghi et al., 2014). Toca 511 delivery is currently undertaking a phase 2 clinical trial in the hopes of eradicating glioblastoma (Caffery et al., 2019).
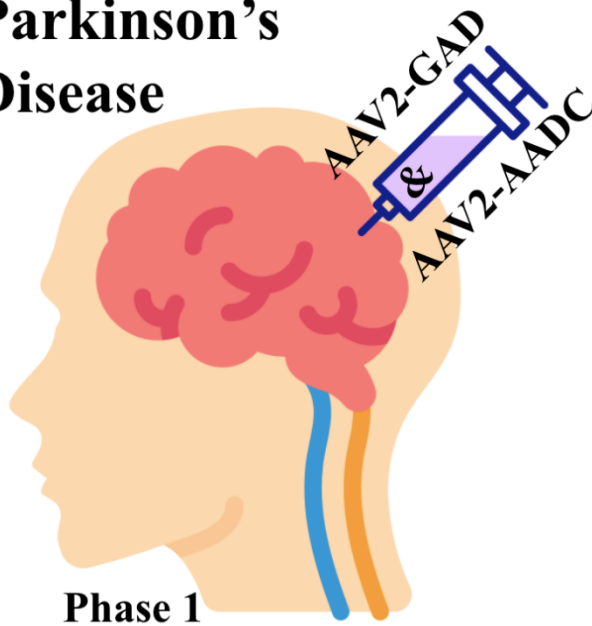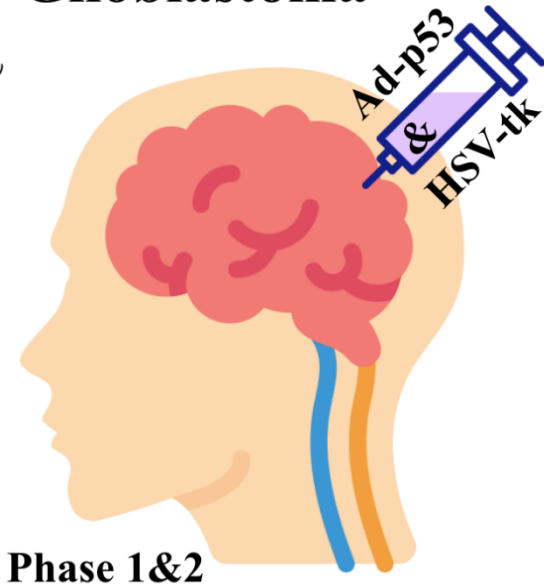
Figure 1. Illustration of two different approaches of each of the two diseases studied. Source: <div>Icons made by Smashicons from www.flaticon.com</div>

Conclusion

Gene therapy has great potential, especially in treating neurological disorders. In this review, we have examined clinical trials of gene therapy for Parkinson's disease and glioblastoma, two currently intractable diseases. There are broadly two gene therapy approaches (GAD and AADC) for Parkinson's disease undergoing clinical trials that are showing special promise. In glioblastoma, adenovirus and retrovirus approaches are being examined, with the delivery of p53 and AdV-tk. Optimizing the vector delivery platform and choice of genetic material to deliver remain challenges that hold potential for greater research.

Acknowledgments

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