Chapter 10

Gene Therapy in Epilepsy

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Abstract

The genetic modification of cell cultures and their transplantation into the brain is an effective ex vivo gene therapy. This transfer of genes via the genetic engineering of viruses or plasmids and subsequent transfection into cells that will express transgenes in the central nervous system (CNS) may allow specific treatment in epileptogenic foci while sparing healthy brain tissue, and minimize the side effects of antiepileptic drug treatment. Prime modification candidates are neuropeptide Y (NPY) and galanin, which are important modulators of neuronal excitability. These neurotransmitters exhibit an inhibitory effect on neuronal activity and provide anticonvulsant effects in animal models. Galanin also exhibits neuroprotective properties. Other modification candidates are adenosine, which acts as an endogenous anticonvulsant, and the glial cell line-derived neurotrophic factor (GDNF), which exerts neuroprotective and anticonvulsive actions. Recombinant adeno-associated viral vectors can release any of these agents because of their neuronal tropism, lack of toxicity, and stable persistence in neurons. This chapter provides an overview of gene therapy methods, and reviews several studies that used neural and non-neuronal cell transplants as a basis for expanding our understanding of diseases that affect the CNS and possible therapeutic alternatives.

Key words Transfection, Viral vectors, Gene therapy, Neuropeptide Y, Galanin, Neural transplant

1 Introduction

Gene therapy is a therapeutic strategy to replace a defective gene with its functional counterpart and restore the operation of a specific cell population. Gene therapy uses nucleic acids tactically, instead of drugs, to correct the pathological state of cells via modification of their genome [1]. Therefore, molecular knowledge of the disease is essential to hit specific targets [2]. Depending on the target cell, two types of gene therapy exist: the first genetically modifies germ cells (i.e., those that are involved in the formation of eggs and sperm), so that this information will be transmitted to offspring. Another type is the somatic gene therapy where the genetic endowment is introduced in non-germ or somatic cells, so genetic modification is not transmitted to offspring [3]. Gene therapy can be performed in vivo or ex vivo. In vivo gene therapy

introduces the genetic material directly into the cells of the organism without prior manipulation in vitro. Its disadvantages are a lower level of control on gene transfer and difficulties in achieving a high degree of tissue specificity. Ex vivo gene therapy removes target cells from the patient, and these cells are isolated and grown in culture medium for transfection. Effectively transfected cells reproduce in culture and are replaced in the patient.

Recent efforts attempted to develop techniques to insert target genes for the treatment of central nervous system (CNS) disorders. The results in animal models treated with gene therapy have created new possibilities in the treatment of multifactorial diseases, such as epilepsy, which led to a rapid development in the manipulation of gene expression in brain cells [4].

Epilepsy is the third most common neurological disease worldwide. It is a CNS disease that is characterized by spontaneous and recurring seizures. Antiepileptic drugs (AEDs) are the first treatment choice, but these drugs are effective in only 60–70% of the individuals [5–7]. Patients who continue to suffer seizures despite more than two changes in their drug regimen are considered for surgical resection of the epileptic focus. However, this option is invasive, it is associated with dysfunctional effects, and only 10% of this population are acceptable candidates for surgical treatment [8]. The death of GABAergic interneurons and plastic changes in the hippocampus have been reported in temporal lobe epilepsy [9]. This cell loss alters the levels of neurotransmitters, in this case dopamine and γ-aminobutyric acid (GABA). Cell therapy for epilepsy proposes the use of replacement cells or ex vivo gene therapy transplantation to administer a substance of interest to the damaged CNS [10]. The objectives of gene therapy in epilepsy are to obtain a sustained anticonvulsant effect (as an antiepileptogenic drug that blocks disease progression, avoids epileptic focus propagation [8], and restores neurotransmitter levels) and the possible integration of transplanted cells into damaged circuits to restore the functions that are lost during the disease progression [11].

2   Gene Therapy Methods

As discussed already, two main methods are used in gene therapy: ex vivo, which involves the removal of tissue cells, in vitro modification using a retroviral vector, and the replacement into the body. In vivo gene therapy consists of the manipulation of the corrective gene within the patient rather than the replication of cultured cells [12].

2.1   Ex Vivo Gene Transfer

The first step in ex vivo gene transfer is obtaining tissue cells from the target organ. Tissue cells are disrupted and seeded in appropriate cell culture conditions in vitro. Cells are transfected with a therapeutic gene using a suitable vector, which should be selected

for its ability to stably and persistently express the exogenous gene. Selected cells are amplified and collected for reimplantation in the organism. Allogeneic cell lines may also be used in cases where the organ or tissue of interest cannot be easily removed or provide cells for in vitro proliferation. The ex vivo method is the most used technique because rejection is minimal [12].

2.2 In Vivo Gene Transfer

This method is based on the systematic management of the genetic construct of interest. DNA may be administered directly, but the use of a vector that facilitates the process of gene transfer and allows entry and intracellular localization, resulting in a functional gene, is a more common method. A noninvasive procedure is also important for the use of specific vectors that allow gene delivery to target cells in a specific organ or tissue. This method is used in cells that are difficult to extract and implant, such as CNS cells [13] (Fig. 1).

2.3 Introduction of the Transgene

A transgene, which is also called a genetic construct, is defined as a set of one or more genes and regulatory sequences that control its expression. The transgene exerts the therapeutic function for gene therapy. Most research on this topic was performed in animal models, but there are clinical trials in humans [14].
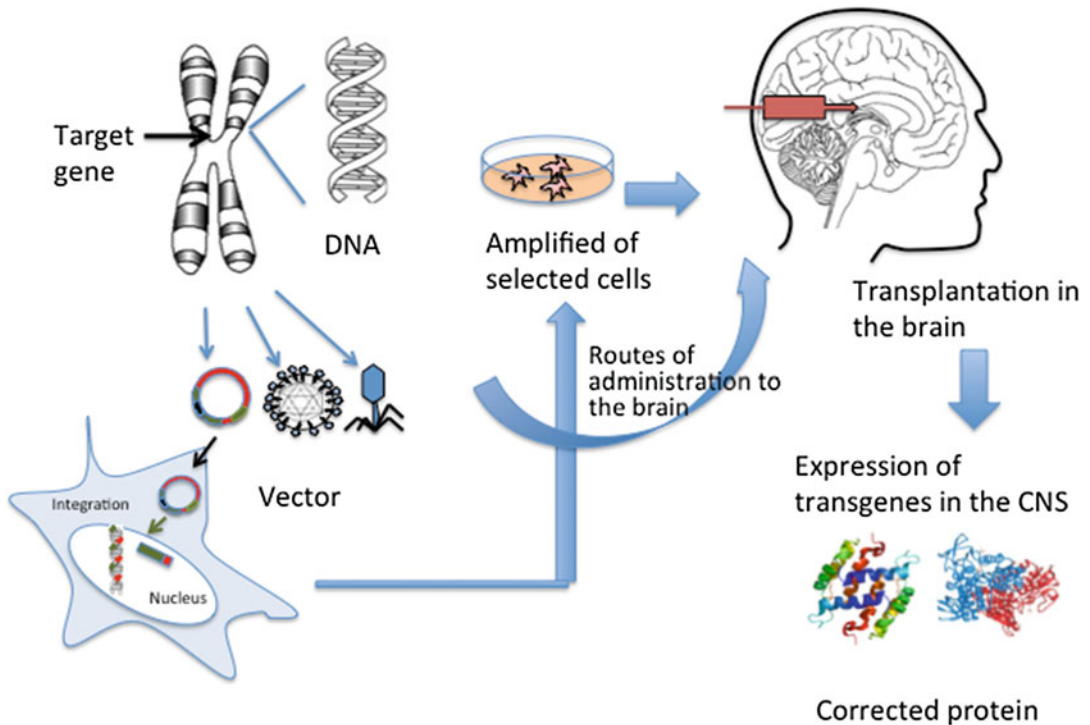

Fig. 1 General model for gene transfer microinjection or misting mediated by different types of vectors. Viral methods: adenovirus, retrovirus, adeno-associated virus, herpes virus; nonviral methods: DNA-liposomes, naked DNA

2.4   Direct Methods of Transfection

2.4.1   Chemical Systems

Calcium phosphate transfection. This technique was first used in the 1960s with good transfection efficiency; it is based on the precipitation of exogenous DNA, with the resulting DNA-Ca^{2+} precipitate entering the cell via endocytosis. Transfection efficiency reaches 10 % of the cells, but it is generally transient. This technique does not cause toxicity in cells, but the transgene expression is low. It is used only in cell cultures and ex vivo applications.

2.4.2   Physical Systems

These techniques involve the injection of DNA into the cell nucleus. This direct introduction of DNA avoids cytoplasmic and lysosomal degradation. Injection is performed using a micromanipulator and an inverted microscope for visualization. Cells with inserted genetic material that survive exhibit high expression efficiency. This technique requires isolated cells, and it is often used ex vivo. The germline gene therapy method injects DNA into embryos, and it is performed in vivo.

Microinjection (*electroporation*). A gene construct is introduced into the cells by an electric shock that causes the formation of pores in their membranes through which the transgenes enter. It is especially suitable for cells with high proliferation rate. However, many cells die as a consequence of the electric shock. Another disadvantage of this technique is that it cannot be used in many cell types.

Naked DNA. It consists in the introduction, usually by intramuscular injection, of DNA in a saline solution or serum to achieve the expression of a transgene. Expression has been observed in thymus, skin, heart muscle, and skeletal muscle. The mechanism of naked DNA uptake is not fully understood but it is postulated to involve the nuclear pore complex. This technique has a low percentage of transfected cells, no integration of the transfected material into the genome once injected, and no replication in the genome. This is a system used for performing gene therapy in vivo [12, 15].

2.4.3   Direct Methods Mediated by Vectors

A wide array of vectors has been used to deliver genes into the nervous system. Several researchers use nonviral vectors composed of naked DNA. These vectors exhibit several problems (Table 1) related to delivery, short-term expression, and immune reactivity against the vector. Viruses may be delivered locally, and many, but not all, are transported to the cell body by axons via retrograde transport mechanisms afetr the infection of nerve terminals. This retrograde transport is particularly attractive for neuropathic pain therapies, as described in the following sections. Though different efficiencies are obtained depending on the type and strain of virus, the herpes simplex virus (HSV) is a highly efficient vehicle for the delivery of exogenous genetic material in humans. Retrograde transport provides means of targeting specific nodes after intradermal infection or other routes of administration.

Table 1
Vector features used in gene therapy

	Retrovirus	Adenovirus	HSV	AAV	Liposomes	Naked DNA
Insert size	8 kb	35 kb	>20 kb	<4 kb	>20 kb	>20 kb
Title (CFU/ml)	107	1011	1010	109	Undefined	Undefined
Integration	Yes	No	No	Uncertain	No	No
Sustained expression	Variable	Transient	Transient	Variable	Transient/variable	Transient
Distribution in vivo	Low	High	High	High	Variable	Muscle
Quiescent cells	Only lentivirus	Yes	Yes	Yes	Yes	Yes
Disadvantages	Insertional mutagenesis/ leukemia	Immunogenic, complex manipulation	Poor ability to insert	limited insert capacity	Immunogenic	Complex administration route

HSV herpes simplex virus, *AAV* adeno-associated virus. Modified from Zorzano, 2003

Nonviral Vectors

The ability of liposomes (structures surrounded by a lipid membrane to resemble eukaryotic cells) to deliver DNA into cells has been known since 1965, but it was not until 1980 that suitable transfection efficiency was achieved.

Cationic liposomes are artificial vesicles prepared with cationic lipids which are particularly adequate to interact with negatively charged DNA. Many lipids are used to form these liposomes, and different lipid mixtures are being tested. These liposomes are recommended for in vitro transfections. Among the advantages of liposomes we can mention the protection of the transgene from degradation until it reaches the nucleus, the unlimited DNA size, the possibility of targeting specific receptors in tissues (e.g., the lipid layer), low immunogenicity, and the ability to transfect nondividing cells. The disadvantages include low transfection efficiency, transient expression, some cellular toxicity, possible inhibition by serum components, and the inability for gene therapy in neurological disorders [13].

Viral Vectors

This methodology began in 1968, when it was discovered that viruses were capable of infecting mammalian cells. The development of packaging cells in 1989, protein composition that is required for functionality is removed from the viral genome, marked an important stage in this methodology, and reduced the degree of virus pathogenicity. Numerous genes and sequences are removed to allow the virus to capture the exogenous DNA. These viral vectors may be used in vivo and ex vivo, and several viruses are used for gene therapy (Table 1, Fig. 2).

Retroviruses. These RNA viruses integrate a relatively large amount (up to 8 kb) of therapeutic genes, but packaging cells are needed to create these vectors. Virus DNA is transferred into the packaging cells using the calcium phosphate technique. A second transduction is performed in which the gene construct of interest is introduced, and the virus is injected into the host; subsequently, it integrates its DNA into the genome to express the inserted gene. No immune response is produced because the host does not express virus proteins. This technique exhibits a high efficiency transduction and expression, and it is a well-studied system. However, this method only infects host cells that are dividing, viral concentrations are low, and integration into the genome is random. Vectors based on more complex genomes are called lentiviruses. Lentiviruses deliver durable transgene expression but require integration. Axonal transport of these vectors is inefficient, and standard retroviruses require cell division for gene delivery. Lentiviruses are integrated into nondividing cells, and they may be pseudotyped with rabies G protein to enhance retrograde delivery [16, 17]. However, these viruses may disrupt normal genes, including tumor suppressor functions, or activate oncogenes [18, 19].
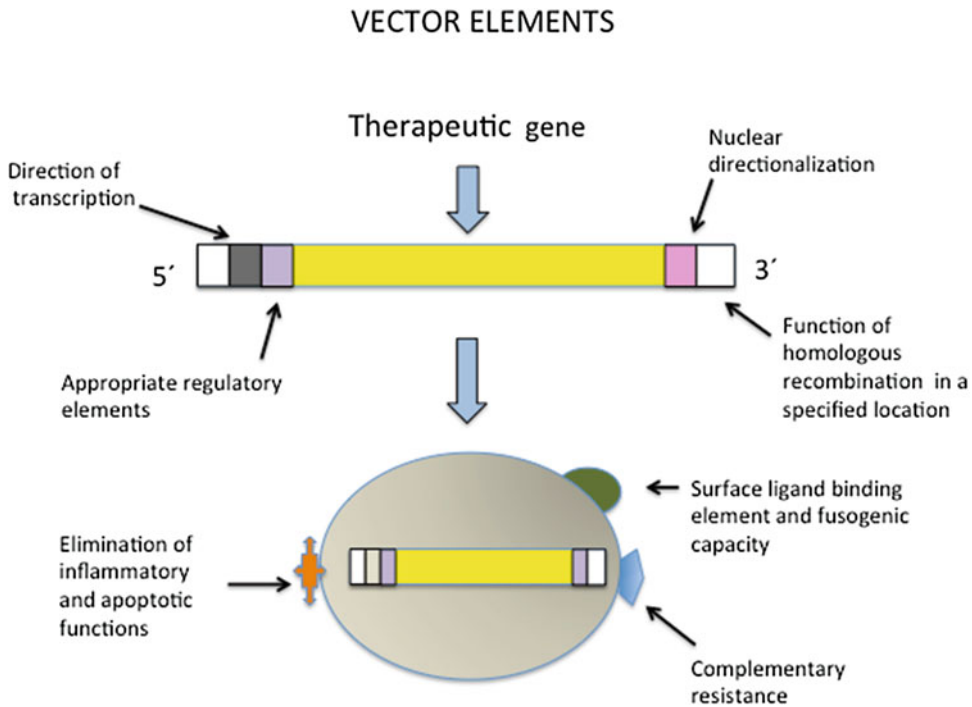
VECTOR ELEMENTS

Fig. 2 Diagram of an ideal gene therapy vector. A vector should possess a number of properties to be used for therapy in an efficient manner. It is also important that the vector does not cause an immune response or other deleterious responses [63]

Vectors that stably persist as episomes are preferred for peripheral nerve applications.

Adenoviruses. Adenoviruses are a family of DNA viruses that cause different infections in humans, mainly in the respiratory tract. Approximately 7.5 kb of exogenous DNA may be inserted into these viruses. Serotype 5 is generally used in gene therapy, but 42 different serotypes infect humans. Integration of the virus genetic material into the host is needed for replication. The transgene is introduced into the genome of the cell, but cellular division of infected cells is not required for replication. The advantage of an adenovirus vector is the high efficiency of transduction and expression of the introduced gene construct, although the expression is transient (a few weeks). Periodic treatments are required, which is a disadvantage because adenoviruses produce cellular and inflammatory immune responses [15].

Adenoviral vectors do not require integration and undergo retrograde transport, but these vectors recruit inflammatory cells because of viral gene expression [20, 21], and they do not persist for a long term in sensory neurons. The immunogenicity of adenoviral vectors has relegated their exploitation to vaccine development and use as oncolytic vectors for cancer treatment.

Adeno-associated virus (*AAV*). AAVs are DNA-containing par-voviruses that require coinfection with an adenovirus to multiply. AAVs are vectors that combine the advantages of retroviral and adenoviral infection, and they can integrate small exogenous DNA (only 5 kb). The primary advantages are that AAVs integrate their DNA into the cell during replication, and transduction (which is highly effective) is stable in the target cell. AAVs also infect divid-ing and nondividing cells, which is highly important for gene ther-apy in vivo. AAVs vectors are not involved in any human disease, and the risk of an immune response to the production of viral pro-teins is minimized. AAVs were efficiently transduced into nerve tissue, retrogradely transported efficiently at least in mice [22–24], achieving long-term transgenes expression. As stated before, one limitation of these vectors is the payload capacity (3–4 kb), and only single moderate-sized genes are generally accommodated. Therefore, virus doses that are required to achieve effective trans-duction are very high compared to other vectors. Further, AAVs vectors do not adequately target specific cell types for infection, especially neurons. Antibodies readily neutralize AAV, and repeat dosing is generally not possible. High vector dosing in the periph-ery and the loading of genomes into cells may induce DNA dam-age responses that lead to inflammation [25, 26]. The disadvantages of AAV have limited the study of this type of vectors, which are not as well studied as retroviruses and adenoviruses [27].

Herpesvirus (*HSV*). HSVs are DNA viruses that can be used to target neurons. The advantage of HSVs is their DNA size, which allows the insertion of various therapeutic genes with regulatory regions. One disadvantage is the need to remove sequences that encode the lytic virus proteins, which kill infected cells.

Some characteristics of the ideal vector for gene therapy include the incorporation and regulated expression of one or more genes for clinical application during an appropriate time, specificity in gene transfer, to be unrecognizable to the immune system, absence of a stable inflammatory response, and easy obtention [12]. The vector designed from HSV allows the insertion of approximately 20 kb, and exhibits a strong neuronal tropism. This vector spreads through the nervous system, which allows a wide distribution of the transfected gene [28]. However, the main limitations are HSV cytotoxicity and its ability to induce cellular immune responses [29]. The HSV-1 variant is used. This vector deletes genes that are involved in viral replication, which reduces neurotoxicity [30]. A specific promoter for tyrosine hydroxylase is also used, which increases the specificity of neuronal transfection [31].

2.5 Genetic Repair Using Oligonucleotides

Oligonucleotides are short sequences of nucleic acids that bind to specific sequences of DNA (triplex-forming DNA) or RNA (RNA-DNA heteroduplex formation). These sequences are complemen-tary to specific sequences of a given messenger RNA (sense

sequence) and form a double-stranded sense-antisense heteroduplex that blocks the translation of the genetic message into the protein. This strategy is used to repair genes that are altered by a well-known point mutation. There are selectively activated cellular mechanisms of DNA repair at the site of the mutation. Specific oligonucleotides are designed adjacent to genomic sequences at the mutation site, and the oligonucleotide carries, on one end, an agent that is capable of damaging the DNA by forming a covalent bond with the nucleotide responsible for the mutation. Selective nucleotide mutation triggers cellular DNA repair processes, which leads to structural and functional gene correction [1].

3   Route of Administration to the Brain

The genetic modification of cell cultures and transplantation into the brain is an effective ex vivo gene therapy. The transfer of genes via genetic engineering of viruses or plasmids and subsequent transfection into cells expresses transgenes in the CNS [32]. The first treatment emerged from adenosine deaminase deficiency. Gene therapy via the intracerebral delivery of a combination of genes was the best option for the treatment of certain CNS disorders, since the blood-brain barrier hindered the access to the CNS [32]. Intranasal administration is a viable option for the expression of transgenes in neural cells. This method achieved CP10PK HSV gene transfection in hippocampal neurons using this same virus as vehicle. However, the level of transgene expression was limited because this route of administration is not the most suitable for therapies that require transfection in specific areas of the brain, unless the vectors are developed with promoters [33]. Stereotactic surgery is a more efficient technique for the delivery of therapeutic genes into specific brain areas because high levels of transgene expression are achieved after the injection of a viral vector [34]. This approach is the most common gene delivery method to the brain, having the lowest population of antigen-presenting cells and lymphatic system in the CNS [15]. However, damage to the blood-brain barrier permits penetration of active lymphocytes and immune response cells, and this transfer technique must be redefined. Other factors should be considered when gene delivery is successful, such as the level and stability of transgene expression and the ability to regulate the expression of the transgene [35]. Lentiviral vectors are used in gene therapy because of their ability to integrate up to a 9 kb chromosome of the host and to transfect most brain cell types, which facilitates the expression of transgenes [15, 35].

Primate and human immunodeficiency viruses (HIVs) belong to the lentivirus family. Therefore, it is important to consider extreme biosecurity measures compared to other viral vectors, to prevent recombination events that could generate a virus capable of replication.

Biosecurity measures include the elimination of virulence genes in packaging plasmids, the introduction of genes involved in capsid assembly using separate plasmids to reduce the possibility of recombination, and the induction of vector auto-inactivation to suppress viral transcriptional activity [27].

The use of recombinant adenovirus (rAAV) vectors for the delivery of transgenes in the brain does not produce toxicity. These vectors also elicit a low immune response and exhibit efficient cell transduction in the brain in animal models. These adenovirus serotypes were isolated from humans and primates, and some of the rAAVs that were cloned and packaged into recombinant vectors exhibited tropism for neuronal types and various brain areas [36]. Injection of high concentrations of viral particles to the brain promoted transgene expression and dissemination, and achieved effective cell transduction [37]. The combined use of rAAV2 vectors and stereotactic surgery achieved transfection in selective foci, such as the hilum or hippocampal CA1 areas [38]. Several rAAV serotypes were characterized recently, and these vectors primarily exhibited neuronal tropism. Therefore, it is important to continue the development of vectors that are more efficient and exhibit more tropism for glial cells, including improved expression cassettes, greater storage capacity, and the characterization of specific promoters for greater expression in certain cell types. Several promoters were used to restrict rAAV expression in melanin-concentrating neurons in the hypothalamus. However, promoters have not been isolated yet, and expression is restricted to neuronal subclasses in the hippocampus, such as hilar GABAergic neurons or principal neurons in the dentate gyrus. This limitation is a particularly difficult challenge for rAAV vectors because of the promoter activity contained within the inverted terminal repeats [39]. The development of treatments for each neurological disorder is important to optimize the efficiency of the systems for gene delivery.

4   Gene Therapy Strategies for Epilepsy

Although a large part of the epilepsies are of genetic origin, genetic targets are not the best candidates for gene therapy. A single mutant gene rarely causes the disease since epilepsy is the result of the inheritance of two or more susceptibility genes [40]. In these cases, the pathology often affects a large part of the brain, which would require widespread gene transfer. These features pose two big obstacles for gene therapy. The first obstacle is the need to transfer multiple genes into diseased cells, and most viral vectors (with the notable exception of HSV) have a small genome that hosts only one gene at a time. The second obstacle is the need for widespread expression of the therapeutic gene(s): currently available gene

therapy methods mostly provide localized effects. These reasons suggest that focal epilepsies, particularly temporal lobe epilepsy, are better candidates for gene therapy. In these cases, seizures often originate in a restricted brain area, which allows local delivery of the vector using stereotaxic surgery. Epilepsy caused by focal lesions often has an identifiable cause from a damaging insult (e.g., head trauma, status epilepticus (SE), stroke, brain infection), which sets a cascade of neurobiological events in motion. The development and extension of tissue that is capable of generating spontaneous seizures would result in the development of an epileptic condition and/or the progression of the epilepsy.

Epileptogenesis (i.e., the transformation of a normal brain into an epileptic brain) in humans and animals is associated with progressive pathological abnormalities, such as cell death (the most prominent is a loss of neurons in the hippocampus termed "hippocampal sclerosis"), axonal and dendritic plasticity, neurogenesis, neuroinflammation, and alterations in ion channels and synaptic properties. The physiopathology of epilepsy is well studied in animal models and surgically resected tissue, and several candidate genes have been identified as potential therapeutic targets [41]. Gene therapy allows specific targeting of the epileptogenic region, which spares the surrounding healthy tissue and minimizes the side effects of antiepileptic drug treatment.

4.1 Gene Therapy in Animal Models of Epilepsy

Research on gene therapy for epilepsy was conducted essentially in several types of models, primarily chemical convulsant (pilocarpine or kainate) evoked SE and electrical stimulation (electrical kindling). SE models induce an epileptogenic insult that is followed by a latency period during which the animals are apparently well, followed by spontaneous recurrent seizures (SRSs), i.e., epilepsy. This situation closely mimics the situation in humans with acquired structural epilepsies [42]. SE models allow the exploration of interventions at different levels: antiepileptogenic (prevention of development of epilepsy in subjects who are at risk after an epileptogenic insult), antiseizure (reduction of frequency and/or severity of seizures), disease-modifying (alteration of the natural history of the disease) and repair of the affected regions, where cells may be modified to produce inhibitory neurotransmitters, such as GABA.

Increased GABA levels in the epileptogenic area are favorable on physiological behaviors because they reduce neuronal excitability and the occurrence of seizures. Löscher et al. [43] observed these effects following the transplantation of fetal GABAergic neurons in the substantia nigra, which transiently decreased seizures in the kindling model in rats [41, 43]. This observation led to the development of the transfection of glutamate decarboxylase (GAD) in in vitro and in vivo models to increase GABA levels (Table 2). Ruppert et al. [44] used a retroviral expression system to obtain complementary DNA (cDNA) and induce the expression of different GAD isoforms in rat

Table 2
Gene therapy used in epilepsy models

Animal model	Gene therapy	Description	Reference
Tetanus toxin into the motor cortex	Optogenetic strategy In vivo Ex vivo	Overexpress the light-activated chloride-pump, halorhodopsin, in pyramidal cells	Walker et al. (2013) [64], Tønnesen et al. (2009) [65]
Kainate-induced seizures	Expression of microRNA targeting adenosine kinase (miR-ADK) In vivo	Silencing of ADK expression using an alternative RNA interference-mediated mechanism in astrocytes	Young et al. (2014) [66]
Kainate-induced seizures	AAV-GS and AAV-EAAT2. In vivo	Overexpression of glutamine synthetase (GS) or excitatory amino acid transporter 2 (EAAT2) Synthesis of GABA	Young et al. (2014) [66]
Limbic kindling	GABAergic cell transfer (GAD 65 and GAD 67)	Reduced the incidence of severe limbic motor seizures, by granule cell stimulation Expression of GAD 65 and 67 Transient increased GABA levels	Gernert et al. (2002) [46]
Kindling	AAV-mediated delivery of an antisense RNA specific to NMDAR1	Influence NMDA receptor function both in vitro and in vivo	Haberman et al. (2002) [51]
Pilocarpine-induced status	Replication-defective HSV-1 vector expressing FGF-2 and BDNF	Attenuated the ongoing cell loss, favored the proliferation of early progenitors, and led to the production of cells that entered the neuronal lineage of differentiation	Simonato and Zucchini (2010) [56], Simonato et al. (2006) [57]
Kainic-induced epilepsy	NSCs expanded from E14 MGE	Reduced frequency, duration and severity of seizures 3 months after graft	Waldau et al. (2010) [67]
Kindled seizures	AAV Vector-derived NPY	Reduced the generalization of seizures from their site of onset, delayed acquisition of fully kindled seizures, and afforded neuroprotection	Noe et al. (2007) [68]

AAV adeno-associated virus vectors, *NMDAR1* N-methyl-d-aspartic acid receptor 1, *RNA* ribonucleic acid, *NSC* neural stem cell, *MGE* medial ganglionic eminence

fibroblasts. These experiments increased enzymatic synthesis and GABA activity, which was released into the surrounding medium by the modified cells. These results demonstrated that the expression of GAD from cDNA was effective for the synthesis of GABA in transplanted cells although not in cells of neuronal origin [44]. Sacchettoni et al. [45] used Moloney murine leukemia virus expressing the GAD-67 enzyme under the control of the GFAP promoter as a replication-defective vector. The clones were transfected into fibroblast cell lines and astrocytes and both expressed functional enzymes. However, only fibroblasts produced GABA in the extracellular medium. Gernert et al. [46] transplanted immortalized cortical neurons with genetically modified GAD-65 into the piriform cortex of rats with amygdala kindling. The GABA-producing cells implanted in potential brain targets enhanced the seizure threshold and augmented the latency to the first generalized convulsion during the kindling process [46]. Liu et al. [47] used vectors derived from human foamy virus (HFV) to transfect the cDNA of a specific GAD isoform into primary cultures of hippocampal neurons, which may have a potential therapeutic value in the treatment of neurological diseases. Another study investigated kainic acid-induced seizures (5 mg/kg, stage V in Racine scale) in rats that previously received transplants of a cell line transfected with human cDNA encoding GAD67 (M213-2O CL4) into the substantia nigra. The results demonstrated that the group transplanted with M213-2O CL4 cells required a higher dose of kainic acid to induce seizure activity, whereas the latency to convulsion increased, and the behavioral manifestation was reduced compared to rats with untransfected cell transplantation [48].

One target for inhibitory mechanisms is the GABA$_A$ receptor, which is the main receptor subtype for the principal inhibitory neurotransmitter. This receptor is a pentameric chloride-permeable channel. The expression of GABA$_A$ alpha-1 subunits is decreased and alpha-4 subunits expression is increased in the granule cells of the hippocampus of epileptic rats, which generates receptors that rapidly desensitize and favor the generation of seizures. Raol et al. [49] used an AVV to achieve the expression of the GABRA1 gene (GABA receptor subunit alpha-1) under control of the GABRA4 promoter (GABA receptor subunit alpha-4). The vector was injected into the dentate gyrus of rats, and SE was produced using pilocarpine. The results revealed an increased expression of GABRA1 mRNA and protein after 2 weeks of SE, and also a three-fold increase during the interictal period and a decrease in the number of rats that developed spontaneous seizures by 60% during the first 4 weeks after SE. These results demonstrate the importance of the composition of GABA receptor subunits for the development of circuits and epilepsy, and the role of GABRA1 expression in hippocampal inhibitory function [49]. The *N*-methyl-d-aspartate (NMDA) receptor also provides a potential target for gene therapy in focal seizures. During et al. [50] constructed an

AAV to synthesize the NR1 subunit of the NMDA receptor. Gene expression persisted for at least 5 months after AAV administration, and it was associated with a strong antiseizure activity and neuroprotective effect in a mouse model of kainate-induced epilepsy and stroke.

Haberman et al. [51] cloned a cDNA fragment (729 bp) of the NMDAR1 gene (NMDA receptor) in an antisense orientation into an AAV that contained a promoter regulated by tetracycline (AAV-TTAK-NMDAR1). The vector was used for the transfection of a primary culture of cortical neurons. The decreased expression of NMDA receptors significantly reduced the evoked currents [51].

Neuropeptides are important modulators of neuronal activity in the mammalian CNS. Neuropeptide Y (NPY) is a widely distributed polypeptide in the CNS, and it has become a focus of attention in the epilepsy field because of the changes in the expression of this protein and its receptors in brain regions that are involved in seizure initiation and propagation. Hippocampal interneurons and NPY-overexpressing axons are observed in patients with intractable temporal lobe epilepsy. The expression of NPY Y2 receptors on presynaptic neurons and pyramidal granular cells inhibits glutamate release and the presence of seizures. Richichi et al. [52] designed a rAAV vector to overexpress rat NPY in neurons and obtained an increase of the expression of this protein in the hippocampus, a reduction in kainic acid-induced seizures and an increase in the latency of seizures.

Cholecystokinin (CCK) was first discovered in the gastrointestinal tract, although it is one of the most abundant neuropeptides in the CNS. This neuropeptide was identified as a central regulator of neuronal circuits, and CCK and its receptors are implicated in the neurobiology of feeding, memory, nociception, exploratory behavior, and anticonvulsant activity. CCK is also associated with neuropsychiatric disorders. Zhang et al. [53] evaluated the potential for CNS gene transfer using lipofectin-mediated plasmids encoding CCK. Intracerebroventricular transfection was performed in rats with audiogenic seizures, and a high analgesic response to peripheral electrical stimulation was observed. Previous studies demonstrated that low CCK levels may vary the neurochemistry of audiogenic seizures in rats and underlie the high analgesic responses pursuant to its anti-opioid properties. CCK expression corrected the development of seizure susceptibility for 1 week. These results suggest that gene transfer mediated by lipofectin may be useful in studies of brain function, modification of behavior, and gene therapy for the CNS. This strategy may result in the transient expression of a foreign gene with functional consequences in the adult brain, but it has the advantage of not recombining with the endogenous virus [54].

The endogenous neuropeptide galanin generally exerts an inhibitory action on the CNS by increasing potassium conductance or

reducing calcium conductance, and the magnitude of this inhibition is sufficient to suppress seizure activity in the hippocampus. Haberman et al. [51] proposed a gene therapy for temporal lobe epilepsy using the transfection of constructs with fibronectin and galanin adenovirus (AAV-FIB-GAL). AAV-FIB-GAL vectors protected the hippocampal hilar neurons from kainic acid-induced cell death, but unilateral secretion did not alter the course of electrographic seizures after kainic acid administration [51]. Other authors achieved significantly attenuated seizures induced by kainic acid using the same constructs (AAV-FIB-GAL) and prevented the electrographic seizure activity, which abolished limbic seizure activity and the bilateral constitutive secretion of galanin in the rat piriform cortex. These studies demonstrated that the modulation of this gene influenced the behavior of limbic seizure activity [55].

The flexibility to transfect exogenous genes achieving protein expression for the treatment of disease, such as protein ICP10PK of herpes simplex virus type 2 (HSV-2), is one advantage of gene therapy. This protein inhibits caspase-dependent apoptosis and protects against several neurotoxic stimuli, including viral infection, treatment with an inhibitor of protein kinase C, osmolar environment disruption, loss of carrier in trophic growth, and excitotoxicity. Notably, neurons that survived gene therapy retained their synaptic function [55].

Laing et al. [33] demonstrated that gene transfection of ICP10PK using HSV2 as a vehicle had antiapoptotic activity in rat hippocampal cultures and mice with kainic acid-induced seizures. This method may be used to target neurons that are inaccessible to surgical techniques. ICP10PK-mediated protection is likely involved in the inhibition of reactive oxygen species (ROS), astrogliosis, and microglial activation induced by kainic acid.

Genetic interventions have attempted to prevent epilepsy after an insult based on the hypothesis that impairment in the levels of neurotrophic factors (NTFs), such as FGF-2 (fibroblast growth factor-2) and BDNF (brain-derived neurotrophic factor), may be a factor in epilepsy pathogenesis. FGF-2 and BDNF protect neurons from damage. FGF-2 is a potent proliferation factor for neural stem cells, and BDNF favors differentiation into neurons [56].

Simonato et al. [56, 57] used a replication-defective HSV-1 vector expressing FGF-2 and BDNF to test the hypothesis that local supplementation of these NTFs would attenuate seizure-induced damage and inhibit epileptogenesis. This vector was injected into one damaged hippocampus 4 days after a chemically induced SE in rats (i.e., during latency and after the establishment of damage). These conditions are similar to a person who is in the latency period after the occurrence of an epileptogenic insult and preceding the onset of spontaneous seizures [58]. Expression of the transgenes was bilateral (because of the retrograde transport of the vector to the contralateral hippocampus) and transient (approximately 2 weeks).

However, short-term expression is an advantage in these specific settings because NTFs trigger plastic changes that remain detectable when the vectors are no longer expressed, and their long-term expression may be detrimental for brain function. Therefore, this approach allowed modification of the microenvironment via the generation of cells that were capable of constitutively, but transiently, secreting FGF-2 and BDNF. This treatment slightly attenuated the ongoing cell loss, favored the proliferation of early progenitors, and led to the production of cells that entered the neuronal lineage of differentiation. All untreated animals displayed hippocampal sclerosis and spontaneous seizures 1 month after SE. These studies provide evidence that gene therapy is an option in epilepsy prevention in at-risk patients. The disease-modifying effect was striking, and the result may be interpreted as truly antiepileptogenic.

Injuries, such as head trauma, ischemia, tumors, and neuroinfections, also generate epilepsy. Brain areas damaged by stroke and seizures express high levels of heat shock protein 72 kDa (HSP72). This protein is a marker of stress, but its participation in the survival of neurons after damage is controversial. Yenari et al. [32] found a neuroprotective effect by inducing the expression of the HSP72 protein in areas damaged by stroke, inhibiting the development of seizures. The vectors used for the transfection of an animal model of kainate-induced focal cerebral ischemia were constructed from HSV.

4.2 Gene Therapy in the Clinic

The process used to develop new gene therapies relies upon experimental validation in model systems. In the case of the epilepsies, the establishment of models has itself been hindered by clinical and genetic heterogeneity. In fact, due to the numerous ways in which recurrent seizures arise and the different types of seizures that can manifest, epilepsy may be considered as a group of diseases, each one potentially requiring different models and strategies for development of maximally effective treatment. Until now, antiepileptic drugs have been devised primarily through seizure models in rodents. On the other hand, preclinical results of Phase I to Phase III clinical trials are highly encouraging and demonstrate that gene therapy does not present a general increase in risk factors associated with the technique compared to other surgical methods for other neurological disorders, including Alzheimer's, Parkinson's, and metabolic diseases [59]. Given the complexity of the pathophysiology of epilepsy, patients with partial epilepsies selected for surgical resection of the epileptogenic area are ideal candidates for gene therapy: the pathology of their illness is focal; the optimal medical treatment has failed to produce the desired results; and the success of surgery in leading centers (>70% seizure-free at 1 year) supports the idea that local and sustained release of an inhibitory molecule might be sufficient to "silence" hyperactivity. In a certain way, tissue resection represents the most extreme form of cellular "silencing," and gene therapy may provide a realistic alternative [60–62].

Data from in vivo experiments using AAV vectors suggest that this method of gene delivery is a more practicable approach to progress to clinical trials. An early proof of principle study demonstrated that gene transfer using AAV vectors in resected human tissue slices resulted in an appreciable level of transduction of cells in the epileptic tissue [61]. No clinical trial has been conducted using this technology. However, the therapeutic approach in epilepsy is directed to interrupt abnormal seizure activity rather than reintroduce a cell population that was lost, which was often the focus of gene therapy for neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases [59, 63–65].

In summary, the experimental and clinical data from other neurological diseases demonstrate the feasibility of gene therapy for epilepsy [66–68]. The genetic modification of cell cultures and transplantation into the brain is an effective ex vivo gene therapy. This transfer of genes using the genetic engineering of viruses or plasmids and subsequent transfection into cells achieves the expression of transgenes in the CNS. Cell therapy and ex vivo gene therapy have increased our knowledge of plasticity mechanisms and the factors that promote cellular integration in the CNS. Basic and clinical studies suggest that temporal lobe epilepsy, which is clearly refractory to traditional pharmacological approaches, is an ideal candidate for gene therapy, which will significantly impact disease management in the coming decade. There is no doubt that accurate verification of safety and scale-up studies are needed before beginning studies in humans, but gene therapy experience in humans with other diseases is encouraging [42].

Acknowledgments

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