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GENE THERAPY AND GENE DELIVERY TO THE BRAIN USING VIRAL VECTORS

GEN TERAPİSİ VE VİRAL VEKTÖRLERİN KULLANIMI İLE BEYNE GEN NAKLİ

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Abstract

Treating monogenic disorders via gene therapy although still considered experimental by some, has becoming a more accepted method lately especially in these last 10 years with a number of recent clinical successes. Genetic modifications are becoming easier to perform with the progressing technology and discovery of new techniques such as the Clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR associated protein 9 (Cas9) methods which can modify DNA with great ease and accuracy. Gene therapy is a powerful technique with huge potential to treat psychiatric and neurodegenerative disorders including Alzheimer's and Parkinson's disease. Gene therapy is simple in principle, which is corrective genetic material is sent into cells and the disease is cured by ending the problem at its source. Viral and non-viral vectors which are used for the delivery of the desired genes to the targeted cells are briefly listed and explained. Unlike viral vectors, non-viral vectors don't cause an immune response but their pretty low transfer rate makes them rather less interesting for research. Viral vectors of adenoviruses, adeno-associated viruses, retroviruses with its subclass of lentiviruses and herpes viruses are compared with their advantages and disadvantages related to usage in brain and CNS treatment of our topic. Neurotrophic factors (NTFs) have important roles in brain and nervous tissue. Delivering NTFs via viral vectors for treating neurodegenerative diseases is a promising approach. Providing information about principles, methods, hurdles and clinical applications of gene therapy with its historic background to present it with its all basic details and therapeutic effects it can provide to problems related to brain are aimed in this writing.

Keywords: Gene Therapy, Viral Vectors, Brain

Özet

Tek bir gene bağlı hastalıkların gen terapisi ile tedavisi hala bir kısım tarafından deneysel olarak nitelendirilse de özellikle bu son 10 yıldaki en son klinik başarılar ile gittikçe daha fazla kabul gören bir yöntem haline gelmektedir. DNA'yı harika bir kolaylıkla ve hassasiyetle modifiye edebildiğimiz clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR associated protein 9 (Cas9) metodu gibi yeni tekniklerin keşfi ve ilerleyen teknoloji ile genetik modifikasyonları uygulamak daha kolay bir hale gelmektedir. Gen terapisi psikiyatrik ve Alzheimer ya da Parkinson hastalığı gibi nörodejeneratif rahatsızlıkları tedavi edebilecek güçlü ve büyük bir potansiyeli olan bir yöntemdir. İyileştirecek olacak genetik malzemenin doğrudan hücrelere yollanması ve rahatsızlığın direkt olarak kaynağından çözümlenmesi, gen terapisinin basit prensipidir. İstenilen genlerin hedef hücrelere taşınması için kullanılan viral ve viral olmayan vektörler listelenmiş ve kısaca açıklanmışlardır. Viral vektörlerin aksine viral olmayan vektörler bağışıklık sistemini tetiklemezler fakat düşük transfer seviyeleri onları araştırmalar için daha az ilgi çekici yapmaktadır. Adenovirüsler, adeno ilişkili virüsler, alt kategorileri olan lentivirüslerle birlikte retrovirüsler ve herpes virüsleri konumuz olan, beyinde ve merkezi sinir sisteminde tedavi amaçlı kullanımlarına ilişkin avantajları ve dezavantajları ile karşılaştırılmıştır. Nörotrofik faktörlerin beyinde ve sinir dokusunda önemli rolleri vardır. Nörodejeneratif rahatsızlıkları tedavi etmek için nörotrofik faktörleri viral vektörler kullanarak iletmek, umut vadeden bir yaklaşım yoludur.

Bu yazıda, bütün temel ayrıntıları ve beyine dair sunabileceği tedavi edici etkileri ile tarihsel arkaplanı da dahil edilerek gen terapisinin prensipleri, metodları, zorlukları ve klinik uygulamaları hakkında bilgi vermek amaçlanmıştır.

Anahtar Kelimeler: *Gen Terapisi, Viral Vektörler, Beyin*

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1. Introduction

Technology is developing with an increasing acceleration and thus enabling scientific progress and increment in our scientific knowledge. With scientific progress our knowledge about the universe is deepening. Biology managed to set its roots and appear as a scientific discipline later than others mainly because it's highly dependent on technology. Biology and its field genetics is one of the most advancing study since 1900 and the start of 21st century.

Genes are the hereditary molecular units in DNA which make up a specific portion of a nucleotide and they encode instructions for making up proteins or RNA (Alberts et al., 2002). Their place in the genome can be located, they can be transcriptased and may have functional regions (Pearson, 2006).

Gene therapy has a huge potential for curing lots of diseases even otherwise incurable psychological disorders related to host's genes as genes responsible for various psychological disorders and human brain's functions are being understood more clearly every day.

Gene therapy is the therapeutic method for treating hereditary or other disorders and diseases caused by some specific genes. Correcting these undesired traits caused by genes with using viral or non-viral vectors and introducing the desired genes are aimed. Faulty genes can also be silenced to turn them off instead of correcting them (La Spada, 2009). Gene therapy seems like an effective alternative for CNS (central nervous system) disorders like Alzheimer's, Parkinson's and Huntington's diseases.

Although being simple in theory gene therapy has various difficulties making it quite hard to perform in practice. Transferring new genes to the host cell is quite hard and has its hurdles. The vectors that are used to transfer genes to the target cells must be efficient at gene delivery and there are various required traits a vector should have to be considered successful (Somia & Verma, 2000). Vectors should be easy to produce to obtain enough vectors for practical usage for the treatment of the patient and to be cost efficient. An ideal vector's side effects like toxic or immunological response should be minimal. Also a successful vector has to express the transgene for a long time to make it have a therapeutic effect. When an immune response is triggered the immune system might block the gene delivery vehicles and even destroy the cured cells as they have been modified with new foreign genes (Verma & Somia, 1997). Immunogenic viruses however may be modified to prevent an immune response. Gene delivery vehicles have to target the right cells to be considered efficient. Being efficient at transferring genes to the attached target cells and having a sustained period of production is desired (Verma & Weitzman, 2005).

Viral vectors are favored as they are far more efficient at attaching to the target cells and transferring their genome to the attached cell than non-viral vectors. Viral vectors however have a capacity of the length of the genome they can carry. Effects of adenoviruses and herpes simplex viruses are not permanent as their genome might be discarded after cell division since they don't integrate their DNA to the host cell's genome. Retroviruses and adeno-associated viruses however, integrate their genome to the host cell's genome.

Genes that are used for transfer might lack the information for splicing variants of the transcript and the critical regulatory sequences for the initiation of transcription. Several regulatory elements such as polyadenylation site for the mRNA that is transcribed or signal sequences are necessary for the transgene to be functional. Regulatory elements like promoters are located on the upstream of the gene that is transcribed. Contrary to constitutive promoters which are always active in the cell in all circumstances, such as viral long terminal repeats (LTR), regulatory elements which are tissue selective confer cell-restricted activity such that the transferred gene is transcribed only in distinct glial cells or neurons. Appropriate transgenes are required to function with the cell type-selective promoters. Promoters can also be modified to regulate the expression of the transferred gene. Some different physiologic factors, like hormones, also effect the expression of the genes. Formation of secondary structure of DNA is facilitated or inhibited by ions so manipulation of ions also can help with regulation of the transgene.

Gene therapy is being used for sicknesses that are caused by single gene mutations like hemophilia or cystic fibrosis. Disorders caused by multiple genes are quite complicated to cure as of now. Clinical trials have been undergone for cystic fibrosis however they were not successful (Crystal, 1995). Two methods of gene therapy exist: somatic cell gene therapy (SCGT) and germline gene therapy (GGT). In SCGT genetic changes that happen in the patient are not passed on to its offspring since all the genetic modifications occur in body cells and not in germ cells (eggs and sperm), gametocytes, gametes or undifferentiated stem cells.

First attempt of gene therapy happened in 1980 by Martin Cline from University of California, Los Angeles (UCLA) who used recombinant DNA (Wade, 1981). Two patients who had β-thalassemia blood disorder which causes serious anemia due to low levels of hemoglobin since beta-globulin gene of the person is faulty or non-existent, underwent the operation. Cline isolated and transformed the β-thalassemia which he extracted from the patients' bone marrow cells (Wirth et al, 2013). Cline, with the intention of increasing the transformed cells' replication ability, included a viral thymidine kinase gene in the viral vector he used. Cline's experiment wasn't successful and he was withdrawn from his

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position at the university and his funding was cut since he didn't take permission from UCLA and he breached National Institutes of Health (NIH) guidelines (Mak, Choma & Green, 2010).

The first successful gene therapy happened in 1990 (Blaese et al., 1995). Lots of unsuccessful trials in its early years made gene therapy seem like an unviable method but successes later achieved gained scientists' attention again (Richards, 2012). Gene therapy became approved for the treatment of lipoprotein lipase deficiency in Europe with the medicine Glybera in 2012 (Ylä-Herttuala, 2012).

1.1. Non-viral vectors

Non-viral vectors are easier to produce and immune response is usually not triggered. Any toxic, if it is caused, is low. Another upside of using non-viral vectors is they can target any type of cell and they do not have a maximum length of genetic material that they can carry however they are less efficient in gene transduction than viral vectors (Nayerossadat et al., 2012).

Naked DNA delivery: Delivery of naked plasmids is the most basic non-viral method. Electroporation or gene gun (Yang et al., 1990) is used to increase the efficiency of naked DNA transfer (Li & Huang, 2000). With electroporation pores on the cell membrane is opened with an electrical pulse and genes are transferred via these pores (Rols et al., 1998). Sonoporation, photoporation and magnetofection are other physical naked DNA transfer methods.

Other than direct physical naked DNA transfer, cationic lipids are used as non-viral vectors. These synthetic vectors made from liposomes don't have a limit to the length of genes they can carry however they might sometimes cause toxic. They are more efficient than naked plasmid transfer still but far less efficient than viruses.

1.2. Viral-vectors

Viral vectors are basically genetically modified viruses to transfer desired genes to the host cell. Usually retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, pox viruses, human foamy viruses and lentiviruses are chosen. Viral vectors have higher chances of causing immune response. Viruses have a limited capacity for carrying genes so they can't transfer DNA with long gene sequences.

Retrovirus vectors: Also known as RNA viruses since they only carry RNA, retroviruses are unable to infect post-mitotic cells like some kidney, brain and liver cells. Retroviruses also carry a reverse transcriptase along with their single stranded RNA. They can only infect still dividing cells (Miller et al., 1990). Maximum length of genes they can carry is 8000 bases. Via membrane fusion the viral capsid with the RNA genome enters the cell after binding to its receptor. By using its reverse transcriptase the retrovirus creates a DNA model of its RNA for the host cell to produce.

Adenovirus vectors: Adenoviruses are the most commonly used gene therapy vectors and they are not highly pathogenic. Maximum length of DNA they can carry is 7500 bases. Changes made by adenoviruses take a long time to take effect as they enter lysogenic cycle and thus those changes on cells might be discarded.

Adeno-associated viruses: Adeno-associated viruses usually don't cause any immune response or sicknesses (Atchison et al., 1965). They can carry up to 5000 base containing DNA. The CFTR gene which is responsible for the disease is 4443 base pairs long so it can fit into adeno-associated viruses and since it doesn't cause harmful side effects it is considered a safe and viable vector for treatment of cystic fibrosis (Colledge & Evans, 1995).

Herpesvirus vectors: Herpes simplex viruses (HSV) which are a member of herpes viruses carry their genetic material as double-stranded DNA. They can carry up to 15000 bases of foreign DNA.

Lentivirus: Lentiviruses are actually a subclass of retroviruses which, unlike other retroviruses, can infect post-mitotic cells meaning that they can infect non-dividing cells. HIV is also a lentivirus.

2. Advantages and Disadvantages of different viral vectors

Compared to other viral vectors, adeno-associated viruses (AAV) are good candidates for CNS gene therapy. AAV vectors are able to target a variety of tissues which are astrocytes, neurons, glial and ependymal cells. Duration of their transgene expression in brain is 6 months long and 6 years long in other tissues of primates. Pathogenesis is minimal as there are no associated pathologies. They are ideal for scalable production as highly pure vector can be produced on large scale (Grieger et al., 2006). Advantages of AAV are that they are nonpathogenic and their expressions are relatively persistent. Their disadvantage is that they can accommodate only 4700 bases of foreign DNA which is rather short.

Retrovirus vectors target neurons and astroglial cells. Their transgene expression's duration is 3 months in brain and 9 months in other tissues of murines. They have some potential pathologies associated with their integration. Retrovirus vectors are moderately scalable production of highly pure vector.

Lentivirus vectors have the advantages of having a persistent expression and being able integrate into host chromosome. Disadvantages of lentivirus vectors are that they can accommodate only 6000-8000 bases of foreign DNA, they are potent human

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pathogens and they are produced in low titers.

Adenovirus vectors' targeted tissues for transduction is neural, astroglial and glioma cells. Duration of their transgene expression is 2 years long in non-brain tissues of primates. Pathogenesis is evident as there is immune response to vector and helper-virus contamination. Helper-virus contamination can be large scale produced. Advantages of adenovirus vectors are that they are episomal, meaning there is no possibility of insertional activation of host genes, they can be grown to high titers (-1010 /mL), they have high levels of expression of the foreign genes and their expression is relatively persistent. Disadvantages of adenovirus vectors are that their genetic manipulation is unwieldy and elicits host immune response.

Herpes-simplex virus (HSV) vectors can target only neurons for transduction and their transgene expression which is 7 months in brain of murine and also unstable, has the shortest duration compared to other vectors. Helper-virus contamination presents pathologies and their scalable production has not achieved yet. Advantages of herpes virus vectors are that they can accommodate up to 15000 bases of foreign DNA, they have high level of expression of foreign genes within hours, they can be concentrated to high titers and they are also episomal.

3. Clinical Applications of Viral Vectors

With the developing vector design technology using gene therapy as treatment for psychological disorders becomes more of a possible option. As our knowledge about the complicated mechanisms of genes such as promoters expands, it seems more possible to manipulate these seemingly modular structures to treat psychiatric disorders (Lesch, 1990). Such treatments can cure rather resistant disorders and cause fewer side effects meaning that the improvements in technology in future will help us applying the modulated version of genes as a therapeutic method.

Adeno-associated virus vectors are a feasible option for targeting central nervous system (Gray, 2012) and they are also considered safe vectors for transferring glutamic acid decarboxylase gene to treat Parkinson's disease as they are not toxic or no other adverse effects were observed later, showing them to be safe for brain (Kaplitt et al., 2007).

Viral vectors however have issues of causing inflammatory and immune response (Cichon et al., 1999). Non-viral, non-immunogenic vectors can be used however, gene delivery using electroporation (Haas et al., 2001) or gene gun (Lo et al., 1994) in vitro, gave poor results of gene transfer. Other non-viral methods such as cationic lipids (Wang et al., 2000) or calcium phosphate (Watanabe et al., 1999) were also not efficient in gene delivery with some toxic caused.

4. Neurotrophic Factors (NTF's)

Multiple aspects of neuronal development including axonal growth, neuronal maintenance, survival and synaptic plasticity are maintained by a range of proteins called Neurotrophic factors. NTFs can slow down or prevent disease process by promoting growth, metabolism and function of neurons in addition of being capable of regrowing damaged neurons (Deister & Schmidt, 2006). There are various neurotrophic factors such as nerve growth factor (NGF), neurotrophin-3 (NT-3), brain-derived neurotrophic factor (BDNF) and NT-4/5 which are neurotrophins, a family of secreted proteins and neurturin (NRTN) and glial cell line-derived neurotrophic factor which belong in superfamily of transforming growth factor beta (TGF-β).

5. Viral Vector Mediated Delivery of NTF's

Viral vectors can be used to supply neurotrophic factors to desired cells via viral-vector mediated gene therapy. Diseased neurons will be supplied with viral vectors which will transduce the diseased neuron for secretion of the therapeutic NTF. For the viral vector mediated delivery of NTFs to be successful early diagnosis of neuronal dysfunction and loss is necessary. Retarding or preventing the progression of the disease has to be the first goal of a gene therapy. Secondly, gene therapy of NTFs should aim enhancing the regeneration of neuronal connections and lost neurons.

For the viral vector mediated delivery of NTFs to have a therapeutic effect for neurodegeneration, cells have to be healthy enough to support the production and release of NTFs at the targeted region. Also signaling and expressions of the related NTFs have to be supported at the targeted region as well.

6. Conclusion

Treating mental disorders which are caused by chemical disruptions of molecular mechanisms of brain or other problems caused by monogenic or even multiple gene errors, is about to become a reality with gene therapy. Delivery of genes into the brain is becoming possible as scientific breakthroughs are happening spontaneously around the world, such as the discovery of the method clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR associated 9 (Cas9) which revolutionized the study of gene editing by increasing the efficiency, speed and pricing of genetic modifying which will also help with improvement of gene therapy (Gori et al., 2015), (Daneshvar, 2015). However before stepping into the complex

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mechanisms and structure of the human brain in order to transfer genes for treatment, there are some steps of precautions and scientific progress that we should not skip in order to be successful. Perhaps achieving a stable transgene expression would be the foremost. Regulation of the transgene along with its induction is important as amount of transgene products are crucial too. Our vector technology has to develop in order to prevent an immune response or toxic upon delivery of vectors into the host and to be able to transfer longer base sequence containing genes. Although cause of some mental disorders such as schizophrenia, bipolar disorder or depression seem to be related to genetics, involvement of other various factors make gene therapy a non-certain treatment for them. However as the effects of various genes in many psychiatric and neurodegenerative disorders is undeniable, sometimes being the sole cause of it, gene therapy is certainly a possible solution which will be applied frequently in future.

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