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Current situation and future of gene therapy for rare diseases

Gengqian Huang

Fujian Vocational College of Bio-engineering, Fuzhou, 350007, China

3243791881@qq.com

Abstract. Rare genetic disorders are rare diseases that have a very low incidence, most of the patients are children, and most of them are caused by genetic defects. Many of these diseases are serious chronic genetic diseases with a small number of patients, low market demand, and high cost of drug research and development, so there is a lack of effective treatment methods, which often threaten life. Gene therapy is an emerging therapeutic approach that uses vectors to introduce genetic material into target cells to treat or prevent rare diseases by correcting or supplementing defective genes. This paper reviews the clinical application of gene therapy for rare diseases such as blood diseases, neurodegenerative diseases and eye diseases. At present, gene therapy still has some technical problems and rare diseases are complicated and cannot be effectively treated. In the future, with further research and overcoming these problems, the application of gene therapy in rare diseases will make continuous breakthroughs and bring good news to mankind.

Keywords: gene therapy, rare diseases, gene editing, gene delivery.

1. Introduction

Rare diseases have a very low incidence and are mostly caused by congenital genetic defects. Most of the patients are children, and many of them are serious chronic diseases with a small number of patients. Different countries, organizations and regions have different definitions of rare diseases [1]. The European Union defines rare diseases as life-threatening or chronic and progressive diseases with a prevalence rate of less than 1/2000 and requiring special intervention [2]. In the *Rare Diseases Act 2022*, the United States defines rare diseases strictly according to their prevalence, that is, the number of patients in the United States is less than 200,000, and the prevalence rate is 1/1500 [2]. The World Health Organization defines rare diseases as having between 0.65% and 1% of the total population for a disease or lesion. However, according to "China Rare Disease Definition Study Report 2021", rare diseases are defined in China as diseases with an incidence less than 1/10000, prevalence less than 1/10000 and a number of patients less than 140,000 among newborn children [2].

There are a wide variety of rare diseases. Now there are about 7,000 rare diseases known. Although a single disease is very rare, the number of patients is about 300 million in the huge global population base, and there are about 20 million Chinese patients, with more than 200,000 new patients every year [3]. 80% of rare diseases are caused by congenital genetic defects and have genetic properties, and 80% of patients develop symptoms in childhood [3]. Families and society are looking forward to new treatment technologies, which is one of the greatest medical challenges of mankind.

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At present, the difficulty of diagnosis and treatment of rare diseases has attracted global attention, and there is a lack of effective treatment methods in the treatment of rare diseases in various countries. This paper reviews the clinical application of gene therapy for rare diseases such as blood diseases, neurodegenerative diseases and eye diseases. The emergence of gene therapy brings hope to patients with rare diseases, and mankind has made a further step in the biggest medical challenge. Gene therapy will promote the development of medical technology in the future, bringing hope to mankind.

2. Gene therapy strategy

Gene therapy is an emerging therapeutic method, which uses vectors to introduce genetic material into target cells to treat or prevent diseases by correcting or supplementing defective genes [3]. It has a good therapeutic prospect in the treatment of cancer and genetic diseases. In 1990, through therapeutic clinical trials in the United States, retrovirus vectors were used to introduce genes with correct coding into patients, restoring gene synthesis in patients, which initiated the first successful gene therapy [4]. Gene therapy for disease requires first identification of pathogenic genes, design of therapeutic genes through pathogenic genes, then specific genes into targeted cells, and finally evaluation of therapeutic efficiency. The selection of gene therapy strategies is crucial for the success of disease treatment. Different treatment strategies can be adopted according to different etiology and pathological changes [5]. To sum up, gene therapy can choose the treatment of diseases according to the changes in etiology and pathology through personalized strategies.

2.1. Gene delivery

Delivery of gene therapy requires vectors. DNA, RNA and protein are easily degraded or inactivated when encountering enzymes. Gene vectors protect DNA, RNA and protein and carry them to overcome multiple intracellular and external obstacles. Vector is particularly important in gene therapy. Vector of gene therapy includes viral vectors and non-viral vectors.

Retroviruses and adeno-associated viruses (AAV) are commonly used viral vectors in gene therapy [2]. Retroviruses belong to a class of RNA viruses, and lentiviruses are a special class of retroviruses that can carry large genes. Nowadays, they are often used as common vectors to deliver genes to hematopoietic stem cells [2]. AAV is a single stranded DNA parvovirus discovered in the 1860s [6]. The AAV vector used for in vivo gene therapy is modified from wild-type AAV. As a result of the natural evolution of the virus, it has high transfection efficiency, and the nucleic acid (DNA or RNA) of the virus itself is transferred to the host cell for replication [3].

With the development of materials and preparation techniques in recent years, non-viral vectors with low price, easy synthesis, easy purification, high transfection rate and low immunogenicity have become the best candidate for gene therapy. Non-viral vectors mainly include liposome vector, polymer vector, inorganic nanoparticle vector, and so on. These vectors can safely transport mRNA across the plasma membrane barrier to antigen-presenting cells [7].

2.2. Gene editing

Gene editing technology can accurately transform DNA sequences at the genome level. Through targeted double-strand breaks generated in DNA, cell DNA repair pathways can be activated to achieve the insertion, knockout and site-specific mutation of target genes, which is a precise therapy in a true sense [8]. Gene editing technology can deactivate or correct mutated genes in the genome, and edit defective genes into normal genes to achieve the purpose of treating diseases [2]. Traditional gene editing technology uses embryonic stem cells and homologous group technology to carry out site-specific modification of the genome [9].

Gene editing technology has a good application prospect in the treatment of genetic diseases and has become the hottest technology in the field of biology, and its application in the field of gene therapy has expanded from single-gene genetic diseases to the treatment of tumors, cardiovascular diseases, infectious diseases and other diseases [7]. However, there are drawbacks such as off-target effects [7].

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2.3. Regulation of geneexpression or gene control

Gene therapy for some diseases requires not only the correct expression of genes, but also the realization of regulated expression of genes [9]. Commonly used small molecule drugs, including tetracycline regulation, sirolimus (rapamycin) regulation, etc., miRNA post-transcriptional regulation has also been applied to gene timing expression design.In recent years, more and more studies have been conducted on gene expression regulated by magnetic and photoregulation [9]. A safe and reliable regulated gene expression system is undoubtedly an important link in gene therapy technology, as well as an important embodiment of increasingly accurate gene therapy [9].

2.4. Gene replacement

In situ correction or replacement of mutant genes in the genome with normal genes is called gene replacement [9]. The main principle of this method is to use the site-specific recombination technology of genes to introduce a gene or expression element with normal function into the cells with abnormal function caused by gene mutation or other parts of the body with gene defects through the carrier, so as to supplement the missing function of the gene through the expression of complete proteins [9]. Theoretically, this method is the most ideal gene therapy strategy, but its disadvantage is that it is only applicable to the treatment of recessive pathogenic genes, such as a variety of hemophilia-related mutated genes, due to the influence of the recessive pathogenic genes [10-11]. For dominant pathogenic genes, functional supplement cannot cover up the influence of gene mutation [9].

2.5. The challenges of gene therapy

Although gene therapy has shown promise in clinical trials, it is also faced with multiple challenges. Compared with traditional drugs, gene therapy is an emerging treatment technology with rapid technological change and involves a variety of ethical and legal issues, especially safety risks [12]. Gene therapy drugs are mostly used to treat rare diseases, tumors and other diseases, and many patients are discouraged by their high price [7]. The production and purification methods of viral vectors are inefficient, and producing enough AAV vectors for clinical trials is a major challenge [3]. The current production and purification system has a series of problems such as difficult scaling up of production process, difficult purification, poor stability, etc. In addition to the complex preparation process of viral vectors, there are also problems such as packaging capacity limitation, insufficient gene expression conversion rate, poor immunogenicity, etc. [3].

Therefore, gene therapy will mainly solve these problems. For different diseases, gene therapy not only needs to be specific and efficient in targeting cells and tissues, but also needs to be able to accurately regulate [2]. Especially for complex diseases such as the expression of insulin genes in diabetes, it is necessary to include receptors that can sense blood sugar levels and determine how much insulin is produced based on blood sugar. And gene therapy needs to be accessible to patients, and the cost of preparation needs to be greatly reduced, from sky-high drugs to affordable drugs for most patients [7]. The development of gene therapy industry still needs to continue to improve the regulatory system, especially in terms of quality control, the establishment of mature third-party testing institutions should be accelerated, and the communication between pharmaceutical companies and drug regulatory agencies should be strengthened [7]. If these problems are solved through the joint efforts of scientific research organizations all over the world in the future, gene therapy, as a new treatment method, will take a historic step in the medical development of the world.

3. Clinical application of gene therapy for rare diseases

Gene therapy has made a lot of progress in the field of medical research, and its effectiveness and safety have been greatly improved. At present, it has achieved good results in the treatment of a variety of diseases. After the success of animal clinical trials, gene therapy began to enter human clinical trials [3]. With the development of diagnostic technology for the treatment of rare disease genes and the progress of gene editing technology, gene therapy has become one of the key research directions for the treatment

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of rare diseases, which uses vectors to introduce genetic material into target cells for repair therapy and "one-time cure".

3.1. Blood diseases

Single-gene diseases affecting proteins and cells in the blood can be treated by gene backup or gene editing [3]. Hemophilia is a rare genetic disorder that causes blood clotting abnormalities. Bleeding from the joints or deep into the muscles can lead to continued dysfunction and even life-threatening bleeding. Therefore, hemophilia, as a single-gene disease, is very suitable to be cured by gene therapy [3]. Gene therapy provides a one-time treatment in which the missing functional gene is introduced so that the cell can synthesize clotting factors again. The functional gene can be introduced into the cell via a vector, usually a viral vector. The first clinical trial using AAV for hemophilia B by delivering clotting factor IX to muscle tissue showed a good safety profile, and the transgene persisted many years after administration [3].

3.2. Neurodegenerative diseases

Most monogenic diseases cause neurological symptoms, and many genetic mutations that cause neurological diseases have been identified [3]. Such as Alzheimer's disease, Parkinson's syndrome, amyotrophic lateral sclerosis, etc. Such neurodegenerative diseases are treated by inducing the formation and growth of neurites. AAV vector can transfer genes into neurons or other nerve cells through one-time administration and has long-term efficacy, providing great advantages for the treatment of central nervous system diseases with AAV [3].

So far, the safety and long-term effectiveness of AAV gene therapy has been confirmed in clinical studies on neurological diseases such as Parkinson's syndrome [13-14]. It shows that gene therapy has great potential in neurodegenerative diseases [3].

3.3. Eye diseases

Aav-mediated gene therapy has good applicability in eye diseases, because scientists have discovered genes responsible for a range of eye diseases. The good anatomy of the eye, with its limited and enclosed physical space, provides unique advantages for local delivery; The blood-eye barrier helps maintain the immune privilege of the eye and limits the immune response [15].

Gene therapy for eye diseases is also of great historical importance. In 2008, three independent groups demonstrated the safety and effectiveness of subretinal injection of AAV-RPE65 [16-17], gene therapy announced its return after more than a decade of silence [18]. Subsequently, the US Food and Drug Administration approved the first AAV gene therapy drug, Luxturna, for the treatment of Leber's congenital black eye syndrome [19].

At present, eye diseases treated with AAV that have entered phase I/II clinical trials include achromatosis (NCT02407678) and retinitis pigmentosa (NCT02556736). No serious complications related to AAV carriers have been found, and some patients have observed therapeutic effects. However, there are differences in visual effects after treatment, and the long-term efficacy is still uncertain. Some patients gradually decrease after reaching their peak treatment efficacy [20-22], possibly due to ongoing retinal degeneration or activation of innate immune responses [3].

3.4. Muscular disorders

Neuromuscular diseases are a group of genetic and acquired diseases that primarily affect one or more components of neuromuscular units, including motor neurons and skeletal muscles. Many neuromuscular diseases, such as Duchenne muscular dystrophy and spinal muscular atrophy, have clear genetic defects and single-gene disease properties, making gene therapy a promising treatment method [3]. AAV gene therapy has achieved remarkable efficacy in clinical trials of infantile spinal muscular atrophy [23]. Fifteen children who received a one-time intravenous infusion of AAV gene therapy had prolonged survival, improved motor function, and some could even walk [3].

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Currently, clinical research is using different AAV serotypes and muscle-specific promoters to treat various neuromuscular diseases, such as Duchenne muscular dystrophy and X-linked myotube myopathy mentioned above [24]. Despite the promise of these gene therapy trials, challenges remain. Some patients develop an immune response to gene therapy, and systemic delivery of high doses of AAV can cause patients to experience transient and acute renal impairment with complement system activation.

3.5. Infectious diseases

Among infectious diseases, gene therapy mainly focuses on refractory diseases such as viral hepatitis, AIDS and SARS [12]. The introduction of protein genes that inhibit viral reproduction and promote viral death or nucleotide sequences that inhibit viral protein synthesis into target cells can reduce viral infection and proliferation in vivo to a certain extent [12]. For example, lentiviral vector-mediated CCR5 antibody expression can effectively inhibit HIV infection [25]. So gene vaccine has become a hot topic in the treatment of infectious diseases.

4. Current status of drug research and development for rare diseases

FDA, EMA, etc. have opened up special channels for rapid approval of orphan drugs, so that orphan drugs have the advantages of a high R&D success rate, short clinical trial cycle, low cost, fast approval, and high market price, which can effectively remedy the defect of fewer target patients; in addition, due to the lack of effective treatment drugs for most rare diseases so far [5]. Moreover, the urgent demand has led to a large development space and low market competition for orphan drugs, which has stimulated the enthusiasm of pharmaceutical companies [5].

5. Conclusion

Due to the complex types and many types of rare diseases [2], many rare diseases with serious diseases are still looking for more effective treatment methods. As an emerging treatment method, gene therapy is expected to solve the dilemma of human beings in the treatment of rare diseases. Gene therapy is still in its early stage and many problems need to be solved, but it can establish personalized strategies. One-time treatment of diseases has a profound impact on the development of rare disease treatment, so there is a lot of research space for gene therapy of rare diseases in the future. Gene therapy is a medical technology with great potential and challenges, which can offer hope and solutions for many diseases that cannot be treated by other methods. Early detection and diagnosis of rare diseases can be realized through genetic diagnosis, and more and more patients with rare diseases are expected to be cured by combining gene editing with personalized medicine. The extensive application of big data and artificial intelligence in medical treatment will also greatly improve the quality of life of patients with rare diseases [5].

With the improvement of the safety and effectiveness of viral vectors, gene therapy has made breakthroughs in many genetic diseases [2], bringing light to patients with rare diseases. The application of gene editing technology makes the original disease-causing genes too large to exceed the packaging limits of viral vectors, so that the gene delivery method cannot be used to make breakthroughs, and the gene therapy research on brain and other technically difficult tissues has also made breakthroughs [2]. However, in order to apply gene therapy to the clinical treatment of more widespread but inherited rare diseases, many problems still need to be solved, such as immune rejection, gRNA recognition range, insertion mutagenesis, genotoxicity caused by off-target effect, ethical issues and high but expensive treatment costs [2]. With the continuous development of future research and continuous solution to problems, it is expected that the application of gene therapy in rare diseases will make continuous breakthroughs in the near future, bringing good news to mankind.

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