Comparison of Classical Gene Therapy and Mrna Drug Therapy

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Abstract: Classical gene therapy, as a new therapeutic means acting on the genome, has become the frontier hot spot and research focus in the field of biotechnology and medicine. Its basic principle is to repair or replace defective genes in the body by directly delivering foreign genes to human cells, so as to achieve the purpose of treating diseases.With the deepening of the research of classical gene therapy, mRNA drug therapy has gradually been summarized as a new type of gene therapy, and occupies an important position.Different from classical gene therapy, mRNA drug therapy is more focused on directly manipulating mRNA molecules to achieve protein production to complete the treatment.Due to the increasing investment in research and application of mRNA drug therapy, classical gene therapy and mRNA drug therapy have become more selective in the treatment of diseases. This article will deeply discuss the differences in development, principle and application direction of the two, explore their respective advantages, and provide ideas and basis for the optimal selection of disease treatment programs in the future.

Keywords:(Classical) Gene Therapy; mRNA Drug Therapy; Delivery Systems; Clinical Applications

1.Introduction

Classical gene therapy (hereinafter referred to as gene therapy) usually involves inserting a healthy copy of a gene into a patient's genome to repair or replace a defective gene in order to treat a disease.The new gene therapy, mRNA drug therapy, bypassed the step of entering the cell nucleus and directly controlled the mRNA to produce proteins to treat disease.The latter has been applied more and more in today's research, but it has not completely replaced the former. It can be seen that these two therapies must have their own advantages and cannot be replaced. This paper discusses the development, principle and application of the two to provide reference for the selection of disease treatment methods.

2. Development and Overview

2.1 Development and Overview of Gene Therapy

The original discovery and research of gene therapy focused on DNA.In 1928, Frederick Griffith infected mice with non-toxic live R bacteria of type I pneumococcus and toxic heat inactivated S bacteria of type II pneumococcus. The mice died of pneumonia infection and concluded: Not only type R must be transformed into type S, but also type I must be transformed into type II. Subsequently, James confirmed and proposed the "transformation principle". Soon, Avery and McCarty also proved that the transformation is caused by DNA.It wasn't until Frederick Griffith discovered in 1952 that bacteriophages could carry DNA from one bacterium to another, that the foundation for in vitro genetic research was laid [1].

Around the 1960s and 1970s, the concept of gene therapy was proposed, in the context of whole-cell development at the time, to test the permanent and stable introduction of foreign DNA into mammalian cells to provide a permanent new genetic function, and then the experimental integration of papillomavirus genetic material into the target cells, And the fact that some of the transferred genes remained effectively expressed in the target cell accelerated clinical development.However, due to technological limitations, it is still unknown how to modify the virus to integrate the expression of foreign genes[2].It was not until the advent of recombinant DNA technology that it was proved that foreign genes could indeed correct genetic defects and disease manifestations in mammalian cells in vitro, and Rogers and Pfordler were the first to genetically modify tobacco Mosaic virus[3],making modifying viral vectors a clinically effective way to correct disease.

After the successful gene modification of

Ashanti De Silva in 1990, gene therapy came into being as a therapeutic method that can achieve long-term expression of therapeutic proteins and tissue-specific expression. As a revolutionary medical means to treat diseases by introducing normal genes into patients to replace or repair defective genes, gene therapy gradually entered the public's attention.

However, from 2009 to 2009, gene therapy has been blocked due to problems such as immunotoxicity. In the past 20 years, it has been clinically applied and successful cases only in SCID and hereditary eye diseases.After 2010, the therapeutic potential of gene therapy was re-tapped. In 10 years, more diseases were approved for gene therapy, and more and more clinical trials were conducted, such as cancer treatment, HIV, and so on.In short, in just a few decades, gene therapy has achieved a great leap from conceptual fantasy to clinical application.

Gene therapy can be divided into germline gene therapy and somatic gene therapy. The main difference lies in whether genetic material or therapy-modified genes are passed on to the next generation after being inserted into the target cell. In order to avoid the ethical problems that may be brought about by germ cells, the current treatment is limited to the latter [1].

Gene therapy mainly consists of genes, gene editing tools and vectors, and since it involves the integration of genes into the genome of human cells, the goal is to achieve long-term or permanent therapeutic effects.

In addition to the initial effect on defective cells to achieve the purpose, with the further development of gene therapy, and explore the use of ribozyme technology and other specific blocking gene expression characteristics, inhibit the expression of harmful genes;The gene of antibody, antigen or cytokine is introduced into the patient to change the patient's immune state;Use the expression products of some genes to metabolize non-toxic or low-toxic nucleotide compounds into special intermediates, which further generate cytotoxicity and lead to cell death;Not limited to diseases with abnormal genes, the use of DNA to promote the body to produce monoclonal antibodies to resist invading pathogens [1]and other new ways to treat.

2.2 Development and Overview of mRNA Drug Therapy

In 1958, Crick proposed for the first time in "Central Rules of Molecular Biology" that RNA participated in the transmission of genetic information and it was confirmed, which led many scholars to increase the research on RNA and devote themselves to exploring the use of RNA molecules to regulate biological pathways to treat diseases.As the intermediate genetic material in the central dogma, mRNA is an unstable intermediate existing between DNA and protein for information transmission [4]. In 1961, mRNA was discovered by Brenner et al. and identified as a messenger for gene translation, but its therapeutic and preventive potential was not discovered at that time.In the first few decades after its discovery, it was not considered as a new drug, because its instability and immunogenicity hindered its development. After that, it took nearly 30 years to establish a therapeutic method using this molecule [5][6].The concept of nucleic acid coding drugs was not proposed until more than three decades ago. In 1989, Malone et al. successfully transfected cationic lipids (N-[1-(2, 3-dioxy) propyl]-N,N, n-trimethylammonium chloride (DOTMA)) by packaging mRNA. Demonstrating that mRNA can be expressed in a wide variety of eukaryotic cells [7]In 1990, by direct injection, mRNA was fully expressed in mouse skeletal muscle cells in vitro for the first time [8], thus tapping the huge research potential of mRNA in the field of therapy and prevention.

mRNA drug therapy is a new therapeutic method that delivers mRNA molecules containing genetic information coding for specific proteins synthesized in vitro to the body, and uses the cell's own mechanism to produce proteins required for treatment.The mRNA does not need to enter the cell nucleus to function; once it reaches the cytoplasm, it is immediately translated.

Strictly speaking, mRNA drug therapy belongs to a type of gene therapy.In terms of carrier integration, gene therapy (here non-classical gene therapy) can be divided into integrated gene therapy and non-integrated gene therapy, the fundamental difference between the two lies in whether foreign genes are integrated into the genome, thus affecting the length of expression time.But the latter is contrary to the original principle of gene therapy.In addition, compared with other non-integrated vectors (such as plasmids), mRNA molecules are widely used in research, especially in vaccine research. Compared with gene therapy on DNA, mRNA

can mediate higher transfection efficiency and longer protein expression time. Secondly, mRNA vaccine also has advantages that DNA vaccine does not have, so it has been gradually summarized as a new type of gene therapy under the background of increasing investment in mRNA drug therapy.

mRNA drug therapy is mainly composed of mRNA molecules and carriers, and there are two types of widely used mRNA, namely traditional non-replicating type and self-expanding type [8] using linear DNA as template and RNA polymerase as raw materials to transcribed from nucleotides.

Since mRNA entering the body as an exogenous substance is easy to trigger immune response and reduce the stability and therapeutic effect of mRNA drugs [9], and mRNA itself has the property of adjuvant, which can improve its own immunogenicity [10], cap structure and tail structure need to be added through modification.

3. Comparison Between Gene Therapy and mRNA Drug Therapy

3.1 Delivery System

3.1.1 Gene therapy delivery system

In gene therapy, the carrier is crucial in the gene delivery process, which can protect the therapeutic genetic material from degradation by nucleases, promote the uptake of genetic material by cells, and safely and effectively deliver the gene to the recipient cell.The vector required for the delivery system should have high payload capacity, high transduction efficiency, little or no genotoxicity, induce the least possible immune response, and have a tendency to target cells [3].

From the viral point of view, the vectors used can be divided into viral vectors and non-viral vectors.Viral carriers include AAV, LV, etc., while non-viral carriers include polymers, lipids and inorganic particles.According to various experiments, viral vectors are dominant, which can effectively overcome various physiological barriers in the body and achieve the targeting of specific types of cells, with high transfection efficiency and tissue targeting, and high delivery efficiency.However, the use of viral vectors has always been controversial, which may cause problems of immunogenicity, so many scholars continue to pursue non-viral vectors with lower cytotoxicity, immunogenicity and mutagenicity and higher stability[12] However, it does not have ideal characteristics, compared with viral vectors, its transfection efficiency is not high, and the duration of expression is also shorter, which drives researchers to continue to explore its optimization program. At present, lipids are widely used in non-viral vectors because of their biodegradability, low toxicity, and the ability to incorporate hydrophilic and hydrophobic substances. Lipid-based gene delivery systems typically use "helper lipids" to promote membrane fusion, cell membrane fluidity, or disruption of bimolecules to improve transfection efficiency, stability,Granules, improved intracellular transport. .

In a word, the choice of the appropriate carrier is the key to the treatment, and the choice of the appropriate carrier depends on In conclusion, the choice of the right vector is the key to treatment, and the choice of the right vector depends on the therapeutic goals and needs, the nature of the desired therapeutic gene, efficiency issues, and safety considerations. Both vector systems are constantly optimized to improve the therapeutic effect.

3.1.2 mRNA drug therapy delivery system

Because mRNA carries anionic negative charge and has a large molecular weight, it cannot enter cells well. At the same time, its stability is not as good as DNA. mRNA existing in the blood is easily degraded by nuclease quickly.Although foreign mRNA has been modified, it may also be recognized by the immune system, triggering immune response, reducing the therapeutic effect and even causing safety problems, which has high requirements on the delivery system.

The therapy has high requirements for the delivery of mRNA molecules to specific tissues and cells. However, the previous delivery system lacks sufficient targeting, which makes the current situation of the delivery system of mRNA drug therapy not optimistic.In addition, compared with gene therapy, mRNA drug therapy has a short expression time, and some long-term treatment diseases need to be repeatedly administered, which leads to challenges in the effectiveness and safety of mRNA.In addition, the high cost is also a big problem in clinical application.

Based on these problems, scientists have found more new ways to deliver mRNA, such as direct delivery of mRNA drugs through nasal inhalation, naked mRNA injection, and optimized carrier encapsulation of mRNA, etc. At this stage, non-viral vectors are the main

carriers, among which LNP is the main application. By changing the proportion and types of cholesterol, auxiliary lipids and cations, researchers can change the particle size of molecules, improve their stability in fluids, and ensure their transmission through tissues and blood vessels. But also adjusts the structure of the lipid in the LNP, changes the targeting property,While improving the delivery efficiency of mRNA to a certain extent, it weakens the immunogenicity, efficiently delivers and releases mRNA into cells, and effectively protects mRNA from degradation [11].

At the same time, in order to solve the problem of targeting, specific ligands or antibodies are modified on the surface of the delivery system;Cost-effective delivery system production methods are also being developed to support clinical scale applications.

3.2 Clinical application

3.2.1 Clinical application of gene therapy

Gene therapy was initially used to treat inherited single-gene diseases that could not be completed by conventional treatment, and later expanded to acquired diseases such as cancer. Although more than 20 gene therapies have been approved now, more than two-thirds of them have been approved for clinical use since 2015.The first clinical trial of gene therapy was conducted by the US National Institutes of Health in 1990, and the number of clinical trials has since increased rapidly around the world. However, in 1999, Jesse Gelsinger died while participating in the clinical trial. Like other experimental medical methods, Was stalled by severe side effects in a small number of patients [13].Long and difficult to get from the lab to the clinic, clinical use of gene therapy flourished again in the early 2000s as the technology advanced and safety was better understood.To date, there have been more than 1,900 clinical trials.

Since the clinical development of gene therapy, the most worrying is its safety and ethical issues. In some cases, gene therapy may not have a therapeutic effect, on the contrary, it may even lead to side effects [14], which also affects the approval of the regulatory system for trials. Safety issues that may arise during the course of the study include:

(1) Insertional mutation

In gene therapy, retroviral vectors are used to integrate genes into the genome of target cells. If the expression of nearby genes is stimulated or disrupted, mutations will occur, which is likely to lead to cancer [15]. The use of non-viral vectors or the removal of strong endogenous elements of retroviruses can reduce the probability of mutation to some extent.

(2) Host immune response

Because some patients carry antibodies and memory cells against AAV, the therapeutic gene carried by AAV can cause immune response in vivo during treatment [16]. Although this immune response will not cause serious consequences, it will affect the therapeutic effect. A variety of approaches have been explored to overcome this pitfall, namely local delivery of AAV to immune-privileged organs. If systemic administration is necessary, a brief period of immunosuppression may help to create a window of opportunity for AAV, while AAV reconstitution may be used to reduce the immune response.

The clinical application of gene therapy is a dynamic field, and regulators are facing the challenge of balancing innovation and safety. Fortunately, the rapid progress of technology has solved most of the safety problems, and more and more successful trials have promoted the progress of this field, which is widely used in cancer, chronic diseases, genetic diseases, etc. [16].

With the success of clinical outcomes comes high costs, not including hospitalization costs and the cost of separating the procedures required to manipulate the cells, which typically cost about $1 million per treatment, and even the cost of hemophilia B reaches the price of $3.5 million per single treatment.Even in rich countries, healthcare systems can't afford it, and in the United States, gene therapy coverage has been limited.In low - and middle-income countries, gene therapy is in direct trouble.

3.2.2 Clinical application of mRNA drug therapy

mRNA drug therapy is mainly used in the field of immunotherapy, and its representative is mRNA vaccine.As early as 2019, there was not much investment in the research of mRNA vaccine. In the new coronavirus epidemic, the research and development of mRNA vaccine has attracted the attention of researchers, hoping to provide a new way for clinically difficult diseases, the first of which is cancer.With the deepening of the understanding of tumor-specific antigen, people are gradually inclined to develop cancer vaccines, but due to

the uncertainty of tumors, the main consideration is to stimulate cellular immunity to achieve the purpose.Clinical trials of several promising drugs are also underway [7]

Although mRNA is optimized on the basis of gene therapy and can skip the nucleus for treatment, it has the same hidden danger of immune response as gene therapy in clinic.

All pose a threat. Exogenous mRNA can be seen as a signal of viral infection, and immune cells can be activated to trigger inflammation through Toll-like receptors. However, this problem can be solved at this stage by reducing the U content of the mRNA or by using certain specific nucleotides.

In addition to this, off-target effects of mRNA may lead to translation in undesired cells or organs, which in turn causes side effects. Therefore, researchers are trying to solve this problem by optimizing the organ or delivery system.

Although mRNA vaccines have shown good safety in the short term, their potential risks are still under study. Some studies suggest that mRNA sequences may be integrated into the host cell genome by reverse transcription mechanism, but this conclusion still needs further exploration and verification.

The production of mRNA drugs requires precise synthesis and purification processes and high-quality materials, and achieving large-scale production and quality control is not a small challenge if clinical needs are to be met.At the same time, the production cost of mRNA drugs is not low, but nowadays, pharmaceutical companies are improving the production technology and improving the utilization of raw materials to reduce costs.With the development and application of mRNA technology, the production cost is further reduced through domestic alternative equipment and raw materials. For example, the domestic consumable production plasmid can compress the cost to ten percent of the original.

4. Summary

In the process of disease treatment, its therapeutic effect and operability are the most concerned issues. In terms of fundamental therapeutic effect, immunotherapy meets the original intention of people to treat diseases, and may provide permanent treatment, including but not limited to genetic diseases; in contrast, although mRNA may also treat genetic diseases, for long-term treatment of diseases, it needs to involve long-term repeated administration, which to some extent increases the probability of inducing immune tolerance.

The operability of these two types of therapies is reflected in the drug delivery system. Compared with the two, because the delivery vector of gene therapy can not find a good balance between viral vectors and non-viral vectors, there are greater safety and effectiveness problems. Therefore, mRNA drug therapy is better in this respect.

Both gene therapy and mRNA drug therapy are revolutionary medical advances. Their development will provide new treatments for many diseases that are difficult to be cured by traditional treatments. They have shown great potential in cancer and other diseases, and these therapies are expected to play an increasingly important role in the future medical field.Effectively screening between the two and selecting the best treatment according to the actual situation of the patient will help further development of the medical field in the future.

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