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Exploring the value of mRNA vaccines

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Abstract. Vaccine development has now been widely used in various medical fields, such as disease prevention, cancer treatment, etc. Therefore, vaccine development has been a hot topic in the medical field in recent years. According to different production methods, vaccines can be divided into three categories: whole-microbial vaccines, subunit vaccines, and genetic vaccines. This article focuses on the design of vaccines using only genetic material, known as genetic vaccines. This type of vaccine is a vaccine that uses specific genetic material to transcribe and translate specific proteins to produce specific antigens. It is more effective and safer. Based on the design and production of mRNA vaccines and their application in treatment in recent years, this article proposes the advancement and shortcomings of mRNA vaccines in the medical field and provides corresponding reference opinions for vaccine development. The original intention of this article is to further improve the effectiveness of mRNA vaccines and thereby benefit society.

Keywords: mRNA Vaccines, Mechanisms, Applications.

1. Introduction

The base sequence in DNA is the main manifestation of biological genetic information. For eukaryotes, DNA is mainly located in chromosomes and synthesizes proteins through ribosomes. Therefore, protein synthesis is not directly determined by DNA but requires an intermediate substance to transmit the genetic information in DNA from chromosomes to ribosomes. This genetic material is messenger RNA (mRNA).

The transmission of genetic information during gene expression includes transcription and translation, so the amino acid sequence of the protein is directly determined by the base sequence of the mRNA. Precursor RNA (pre-mRNA) is formed during the transcription process, of which approximately 25% is mRNA that can be translated into protein, and most of the remainder is non-coding sequences. Because pre-mRNA is not processed, its molecular size will vary greatly, and it is also called heterogeneous nuclear RNA.

Vaccine development has now been widely used in various medical fields, such as disease prevention, cancer treatment, etc. Therefore, vaccine development has been a hot topic in the medical field in recent years. Based on the different production methods, there are three types of vaccines: whole-microbial vaccines, subunit vaccines, and genetic vaccines. Whole microbial method vaccines, resulting in inactivated vaccines, attenuated vaccines, and viral vector vaccines (commonly adenovirus), inactivated vaccines, on the other hand, have the disadvantages of high dosages, a limited immunization cycle, and a single route of immunization. The main disadvantage is that they can occasionally cause antibody-

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dependent enhancement (ADE), which might exacerbate virus infection. A series of adverse events generated by ADE have a significant unfavorable impact on vaccine development. Making an adenovirus vaccine starts with using gene editing to modify the viral into a faulty form. The disadvantage is that antibodies may exist in the human body that can neutralize the adenovirus vector, potentially attacking the vector and decreasing the vaccine's effectiveness. Subunit vaccines can have either protein or sugar subunits. This method's application does not require the entire microbe as a carrier and is relatively safe. Genetic vaccines are obviously vaccines that use specific genetic material to transcribe and translate specific proteins to produce specific antigens. They are more effective and safer. For example, among the new coronavirus vaccines, the RNA vaccine developed by Pfizer of the United States is much more effective than other types of vaccines.

This article will focus on the design of vaccines using only genetic material, known as genetic vaccines. This type of vaccine is a vaccine that uses specific genetic material to transcribe and translate specific proteins to produce specific antigens. It is more effective and safer. For example, among the new coronavirus vaccines, the RNA vaccine developed by the American company Pfizer [1] is much more effective than other types of vaccines. In addition, the most popular among genetic vaccines is the mRNA vaccine [2]. In order for mRNA vaccines to generate an immune response in vivo, antigen-encoded mRNA molecules must be delivered to immune cells, which use the intended mRNA as a template to synthesize foreign proteins that are normally produced by pathogens (e.g., viruses) or cancer cells. An adaptive immune response induced by protein molecules created during the process has resulted in the body's detection of infections or cancer cells. RNA encapsulated in lipid nanoparticles is what makes up mRNA, which protects the strand and facilitates its absorption into cells. Thoroughly studying the mRNA vaccine and putting it into use will have a profound impact on clinical practice.

2. Design and manufacturing of mRNAs

2.1. Transcription process of mRNA

The transcription process [3] is responsible for producing mRNA, when genes are converted into primary mRNA transcripts in the body, RNA polymerase in eukaryotes synthesizes precursor mRNA, which is then processed into mature mRNA. mRNA has a structure similar to DNA in that its genetic information is similarly contained in the nucleotide sequence, which is encoded by a codon and also comprises three ribonucleotides. To ensure mRNA expression in vivo, it is important to boost the mRNA synthesis machinery of eukaryotic cells to finish RNA transcription in vitro. To achieve success in mRNA therapy, optimizing mRNA is crucial.

In eukaryotic cells, mRNA translation involves the building of RNA complexes as well as the synthesis of polyproteins by the ribosome at the start codon. In decoding ribosomes, certain amino acids are created in mRNA, and studies have revealed that there is a certain equilibrium between mRNA degradation and translation [4]. Many structural components have been linked to mRNA degradation in studies, therefore these elements will play a crucial role in the creation of mRNA vaccines. During the developing process, researchers will also aim to modify the structural features that increase mRNA.

2.2. mRNA structure design

The 5' cap is the first thing to optimize in mRNA design. Many mRNA structure optimization algorithms are devoted to optimizing cap analogs, which are positioned at the 5' end of mRNA and have various degrees of methylation [5]. Then there's the poly (A) tail. The tail of mRNA is a dynamic addition, and its length is important in determining the translation impact and protein expression of mRNA. There are also 5'-UTR and 3'-UTR. Although the 3' and 5' UTRs of mRNA do not encode proteins, they are significant in controlling mRNA translation and protein production. UTRs regulate the translational efficiency and stability of mRNAs and play a role in their subcellular localization. The 5'-UTR and 3'-UTR work cooperatively to control protein expression. Codon optimization, the introduction of functional peptides, and the replication process are the primary goals of open reading frame (ORF)

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design. Cap1 is the most popular in mRNA vaccination design, although it requires a high level of competence.

2.3. Chemical formula design

There are two types of RNA that are particularly important in the chemical formula design process. The first is self-amplifying RNA (saRNA) [6] Compared with traditional mRNA, Another type of mRNA molecule, saRNA, has a different structure. The main source of saRNA is alphaviruses and it is constructed by replacing the gene sequence that encodes for viral structural proteins.[7]. Therefore, saRNA can exploit the innate properties of alphaviruses to produce large amounts of proteins of interest in an efficient manner. Followed by circular RNA, non-coding RNA and competing endogenous RNA: Circular RNA (CircRNAs) [8] can serve as protein baits, scaffolds, and recruiters, and exert biological functions by acting as transcriptional regulators, microRNA [9] sponges, and protein templates. Circular mRNAs have a unique structure that enhances their stability, half-life, and resistance to RNase R [10], which is absent and expected from linear mRNAs. An example. In this example we can see that there are footnotes after each author's name and only 5 addresses; the 6th footnote might say, for example, 'Author to whom any correspondence should be addressed.' In addition, acknowledgment of grants or funding, temporary addresses, etc might also be indicated by footnotes.

2.4. Manufacturing

For the manufacturing of mRNA, the synthesis and optimization of RNA are also important links in the production of mRNA vaccines. In vitro conditioning (IVT) [11] mRNA can be achieved by using linearized signal DNA templates or PCR templates, and at least one promoter and corresponding mRNA construction sequence are necessary. The process of obtaining mRNA requires capping, and two methods are usually used: co-transcriptional capping and post-transcriptional capping. It is common for the Poly(A) tail of IVT mRNA to be encoded in the DNA template or attached to the IVT mRNA through enzymatic action. Overhangs occur when poly(A) tail extensions are encoded in the template vector.

There is a Kozak sequence downstream of the 5' end cap upstream of the start codon. The so-called Kozak sequence means that there is a G or A on the third base pair upstream of the ORF start codon in which it participates, and there is a G immediately downstream. However it is not necessary, and its ORF transcription effect is higher. It is speculated that site-directed mutation can be carried out through gene editing technology, and it is believed that the Kozak sequence is known, thereby improving translation efficiency.

2.5. Purification

Because IVT mRNA is mixed with RNA polymerase and DNA template after synthesis, it must be purified, which includes the removal of immune stimulatory impurities such as free ribonucleotides, short mRNA, and DNA template. To obtain high-purity mRNA, the synthesized mRNA can be purified and isolated using commercial purification kits, followed by ethanol or isopropanol precipitation to remove most contaminants, followed by chromatography or elution of gel membrane columns from silica gel to remove components such as proteins and free nucleotides. However, dsRNA impurities [12] cannot be eliminated. A new purification approach, RNase III [13], has been proposed to remove dsRNA contaminants from the transcription reaction fluid, which can considerably lower the immunogenicity of mRNA. To summarize, there are numerous ways for purifying mRNA, which must be chosen based on the research or application aim. Regardless of the purification process used, careful mRNA quality control is required to maximize the benefits of mRNA treatment.

3. Applications of mRNA vaccines

mRNA-based therapy is predicted to become a powerful treatment For various difficult-to-treat diseases, such as infectious diseases, melanoma, pancreatic cancer, colorectal cancer, etc.

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3.1. mRNA therapy based on coding molecules

mRNA-based immunotherapy, which expresses antigens and then initiates an immune response, is defined as an indirect treatment that does not encode therapeutic proteins in the mRNA of viruses or tumor cells. mRNA therapy is a direct strategy to directly treat diseases by delivering functional proteins based on mRNA. These functional proteins mainly include deleted or down-regulated endogenous proteins, functional exogenous proteins or antibodies, and proteins used in gene editing tools.

3.2. Based on mRNA monoclonal antibodies and immunotherapy

The field of biopharmaceuticals has made rapid progress in the field of antibody-based drugs, but the fragile properties of monoclonal antibodies limit their application worldwide. Nucleic acid-encoded monoclonal antibodies, especially those based on mRNA, bring great hope for improving the therapeutic effect of antibodies. Additionally, the authors examine the use of mRNA-based drugs, with a focus on clinical trials of vaccines for the prevention and treatment of infectious diseases and cancer. The most classic example of an mRNA vaccine against infectious diseases is the SARS-CoV-2 mRNA vaccine: SARS-CoV-2 emerged in 2019 and triggered a global pandemic, and the first COVID-19 vaccine was approved for emergency use by the FDA. Authorization. In addition, it also includes influenza virus mRNA vaccine, HIV mRNA vaccine, respiratory syncytial virus (RSV) [14] mRNA vaccine, herpes simplex virus (HSV) [15] mRNA vaccine, varicella-zoster virus (VZV) [16] mRNA vaccine, human cytomegalovirus (HCMV) [17] mRNA vaccine, rabies virus mRNA vaccine, dengue virus mRNA vaccine. Cancer vaccines include; melanoma vaccine, malignant glioma vaccine, acute myeloid leukemia vaccine, renal cell carcinoma vaccine, etc.

3.3. Tolerability and safety of mRNA cancer vaccines

Studies have found that due to their absence in normal tissues, neoepitopes [18] can bypass central tolerance with high immunogenicity and generate an accumulation of genetic mutations in cancer cells. Thus, neoepitopes are utilized to overcome the central tolerance of cancer vaccines and address the problem of tumor heterogeneity. The mRNA vaccine has been found to be safe, with adverse reactions generally ranging from mild to moderate.

3.4. Protein replacement therapy based on mRNA

Protein replacement treatments can be used to replace missing proteins with beneficial proteins in a wide variety of applications. The use of therapeutic methods based on mRNA has become a new pillar of protein replacement therapy. The diseases currently applied include heart disease, lung disease, blood system disease, metabolic disease, cancer, orthopedic disease, Neurogenic diseases, muscle atrophy, etc.

3.5. mRNA encodes peptides and proteins

The functions of peptides and proteins encoded by mRNA are key factors in selecting targeted cell therapy drugs and directly affect the design of mRNA treatments. For cells that require post-translational modifications to assemble into functional types of polypeptides, precise delivery using appropriate protein convertases or endoproteases is required. To perform their functions, proteins must be secreted outside the cell. Therefore, the mRNA needs to be delivered into cells with a natural secretion function, otherwise, the corresponding signal peptide mRNA sequence needs to be inserted near the ORF of the secreted protein.

4. Discussion

Although mRNA therapeutics offer many benefits, current research and development still encounter many problems. In addition to the stability of drug candidates that need to be improved and the technical difficulties associated with targeted delivery that need to be resolved, other major issues include limited expertise and talent, lack of need for specialized infrastructure, excessive capital investment requirements and restrictions, and not being very clear and unified manufacturing practices (GMP). In addition, the current market promotion design of mRNA-based therapies is mainly through

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intramuscular injection. It seems that this trend is unlikely to change dramatically, at least it is not foreseen that there will be no improvement in the near future. Moreover, the largest share of mRNA-based therapies in the global market is in the field of infectious diseases, but it still lacks an absolute advantage in tumors or rare diseases. But in any case, the increasing research and development of RNA-based therapies in different disease areas indicates that this technology has a very wide range of uses and is currently a highly active research field. Advances in biotechnology and the resulting molecular advantages have greatly driven research and development efforts in RNA-based therapeutics. Compared with other treatment modalities, RNA-based therapy not only has a multifunctional delivery platform, but also demonstrates high efficacy, high immunogenicity and low toxicity. As described in this article, RNA-based therapeutics in infectious diseases carry low adverse risks of unintended infection and low potential for insertional mutagenesis. Secondly, the in vivo half-life of mRNA can be adjusted through bioengineering, making different modifications or improving drug delivery methods to make the drug more stable. Third, it is highly transcribable and often degraded by typical cellular activities, thereby reducing the risk of long-term persistence in the body. Fourth, due to the high yield of in vitro transcription reactions, the manufacturing of drug candidates through mRNA technology is cost-effective, rapid and scalable.

There is no doubt that the continuous advancement of mRNA-related technologies in the future will make it widely used in more fields, especially cancer immunotherapy and infectious disease vaccines. For example, technologies such as mRNA-based induction of pluripotent stem cells, mRNA nuclease-assisted design, and protein delivery have all begun to be used in drug development. In the next few years, emerging treatments based on mRNA technology will develop rapidly, such as allergy tolerance therapy, which modulates allergies caused by helper T cells (T helper) and regulatory T cells (Treg) by editing plasmid DNA and mRNA. response; cancer immunotherapy, which enhances or changes the autonomous immune system to better detect and attack cancer cells, or uses microRNA and mRNA to regulate immune-related genes, thereby activating immune responses to fight cancer cells; genome engineering therapy or gene Therapies, including transplantation of normal genes or efficient and precise editing of nucleic acid genomic sequences to replace missing or defective genes, thereby correcting genetic diseases or promoting inactive beneficial mechanisms or pathways, giving them the potential to treat genetic diseases; proteins Alternative therapies, mRNA drugs are considered to offer effective alternatives to gene therapies, particularly protein alternatives, as they avoid the risk of genomic integration and provide robust transient expression.

5. Conclusion

The highly contagious nature of COVID-19 requires new research progress in the development of related vaccines. This article first introduces the concept of mRNA vaccines based on previous research. By introducing the design and manufacturing of mRNA vaccines, it proposes the application of mRNA vaccines in the medical field in recent years. By analyzing the application of mRNA vaccines in medical treatment, this article compares and lists the shortcomings of current mRNA vaccines in treatment, and proposes improvements that need to be made in future research. It is hoped that in the future, scientific workers can gradually improve and overcome the above problems so that mRNA vaccines can benefit all mankind more safely.

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