The Application of mRNA in Treating Cancer

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Abstract

Messenger ribonucleic acid (mRNA) immunity and polypeptides, chimeric antigen receptor T cells (CAR T), deoxyribonucleic acid (DNA) immunity. These drugs have the advantages of simple preparation, low cost, low toxicity, and high biosafety in cancer immunotherapy. It is an up-and-coming drug candidate for tumor treatment. At present, many enterprises have the production technology of mRNA vaccines. International pharmaceutical enterprises for mRNA immunotherapy of cancer are in clinical research and have shown good immune effects in clinical practice.

Keywords: tumor, immunotherapy, mRNA, gene therapy

1. Introduction

Tumor is a disease that has troubled human beings for a long time. Traditional cancer therapies, such as surgery, chemotherapy and radiotherapy, will cause more physical damage to patients. For example, cytotoxic effects induced by chemotherapy and radiotherapy can also lead to the deterioration of patients' condition [1]. Immunotherapy for cancer has become a research hotspot because of its significant advantages such as small side effects, wide range of applications, and little trauma to human body. Immunotherapy for cancer mainly includes peptides, chimeric antigen receptor T cell deoxyribonucleic acid and messenger ribonucleic acid. The latter two belong to gene therapy.

There are serval common approaches applied in the cancer immunotherapy, such as polypeptide immunotherapy, chimeric antigen receptor T-Cell immunotherapy, deoxyribonucleic acid and messenger ribonucleic acid immunotherapy. However, there are some disadvantages of theses approaches. For examples, the drug dose of polypeptide should be frequent and the dosage is high and the raw materials and preparations have high technical barriers. Compared with polypeptides, mRNA therapy can directly act on some mutated pathogenic genes and has stronger immunogenicity, which reduce the drug delivery frequency, the production cycle is shorter, and the cost is lower [2]. CAR-T treatment has high cytotoxicity and many inflammatory reactions. The cytotoxicity of mRNA tumor vaccine is low, and its harm is low after being injected into human body. Different from mRNA therapy, DNA therapy requires the use of viral vectors and entry into the nucleus. It is risky to integrate viral vectors into human genome, and there are potential risks in triggering nucleic acid sensing receptors of innate immune system, genome rearrangement, anti-vector immunity and skeleton effect. mRNA is not only simple in preparation process, but also the reaction is carried out in the cytoplasm and does not enter the nucleus, which making the whole mRNA processing system more bio safe, and the mRNA expression efficiency is more higher [3]. Compared with other immunotherapy methods, the short production cycle of mRNA is considered to be one of its core advantages [4]. Additionally, in principle, mRNA can express any protein type, which means it can be used to treat almost any disease.

These characteristics indicate that mRNA tumor immunoassay is more promising than other immunoassays. Because of these unique advantages, mRNA has been used to express tumor vaccines and tumor-associated antigen [5]. For example, human papillomavirus (HPV) tumor vaccine, breast cancer, renal cell carcinoma and melanoma have been used clinically. Therefore, in this review, we systematically introduced the application of mRNA in cancer therapy and its main production methods.

2. Potential mechanism of mRNA in tumor therapy

mRNA tumor vaccines are nucleic acid vaccines. The main purpose of cancer immunotherapy is to stimulate cell immune response by targeting cancer cells with preferential or restricted expression [6]. mRNA cancer vaccines are injected topically into muscles, under the skin, or into tumors. At the injection site, the dendritic cells educating the T cells to search for cancer cells, the cells that presents antigen, like immune response dendritic cells, present the translated antigen with major histocompatibility complexes I (MHC class I) or major histocompatibility complexes II (MHC class II), trigger the cellular immune response, and produce anti-cancer antibodies that kill cancer cells in the body [7], as shown in Figure 1.

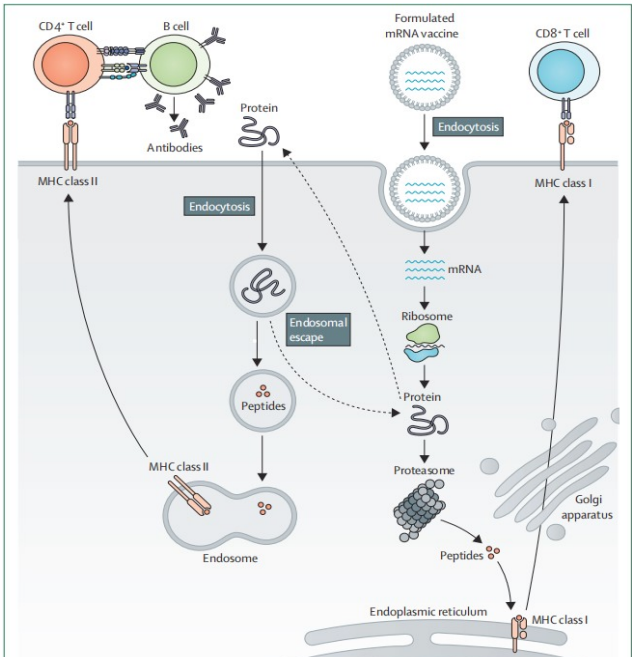

Figure 1. Immune mechanism of mRNA cancer vaccine

3. Production process of mRNA

The preparation of mRNA has a key influence on its successful application in cancer therapy. Based on the action principle of mRNA vaccine described above, many enterprises have realized the industrialization of mRNA, such as the internationally renowned company Sartorius, Danaher BioNTech, Moderna and so on. At present, the main production process of mRNA vaccine is show in Figure 2.

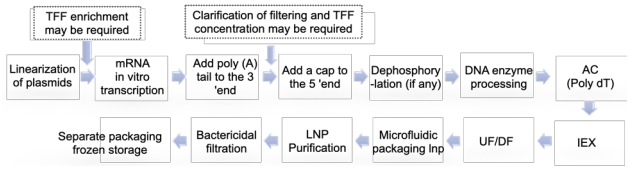

Figure 2. The production process of mRNA

Remarks : AC: Affinity chromatography, UF/DF: Ultrafiltration & Diafiltration, IEX: Ion exchange chromatography

3.1 Preparation of plasmid

As plasmids can copy and replicate their own DNA in the hosts, and are non-viral vectors with simple structure. So they are used to construct eukaryotic expression vectors by recombining foreign genes encoding certain antigens with plasmid DNA vectors. First, retrieve the target gene sequence which controlling tumor or cancer and built the target gene sequence into the vector plasmids. Then construct, enrich and purify the recombinant plasmids.

3.2 Linearization of plasmid

At present, There are two ways to linearize plasmids in vtro transcription(IVT), by enzymolysis or polymerase chain reaction (PCR), the plasmids either can be cleaved to linearized DNA [8].

3.3 Add tail and cap to mRNA

In order to maintain the stability of mRNA and improve the splicing efficiency of mRNA translation, it is necessary to cap and tail onto mRNA during or after mRNA transcription. Cap or tail are also the key signal factor to promote ribosome targeting to mRNA. The capping of mRNA mainly refers to the addition of 7-methylguanosine (m7G) to the first nucleotide of the 5 ′ end, cap structures include three types: Cap 0, Cap 1 and Cap 2. The poly A tail refers to the polyadenylate tail at the 3 ′ end. A new generation of capping is technology Clean cap, which method has more than 94% higher capping efficiency and the toxicity is small [9].

3.4 Delivery of mRNA

In the process of intracellular delivery, mRNA drugs are negatively charged and hydrophilic, so they cannot penetrate the cell membrane. They need to be delivered to the carrier and/or chemically modified to achieve their purpose. With the aid of chemical modification, such as immunogenicity and nuclease, mRNA can be delivered. Lipid nanoparticles (LNP) system has the advantages of easy degradation, promoting cell uptake and internal escape, and protecting nucleic acid from nuclease degradation[10].

4. Research on mRNA based cancer therapy

In 1961, Boczkowski et al. first found that mRNA, to a certain extent, showed an inhibitory effect on tumor in mice [11]. In recent years, remarkable progress has been made in the clinical studies of mRNA, as show in table1.

Table 1. Clinical studied of mrna used as a replacement protein

Name	Payload(for example, antigen or protein)	Disease	Sponsor/ collaborator
BNT141	Secreted IgG antibody	Cancer	BioNTech
BNT331/ GEN1046	BISPECIFIC ANTIBODY PD-L1×1BB	Cancer	Genmab-BioNTech
BNT312/ GEN1042	Bispecific antibody CXD×4-1BB	Solid tumors	Genmab-BioNTech
BNT211	CAR T for CLDN6+tumors	CLDN6+ tumors	BioNTech

The phase II clinical trial of two types of mRNA tumor vaccines, namely, Moderna/Merck's mRNA-4157 and BioNTech/Genentech's BNT122, showed the combination of mRNA-4157 and drug K improved the mPFS of patients with head and neck squamous cell carcinoma to 9.8 months, 100% higher than the first-line standard treatment. The mRNA-4157 contains up to 34 tumor antigens, which is suitable for the treatment of melanoma, NSCLC, HPV bladder cancer, and other types of diseases [12].

BioNTech has entered Phase II clinical practice with BNT111 and HPV16 for melanoma and BNT113 for positive squamous cell carcinoma of the head and neck. BNT111 has related antigens encoding four kinds of melanoma, and it is capable of killing more than 90% of mutant melanoma. It has obtained FDA fast track certification [13].

BioNTech uses the personalized neonatal antigen Specific Cancer vaccine (iNeST) autologous gene, combined with mFOLFIRINOX chemotherapy and the PD-L1 antibody Atezolizumab. Clinical data show that pancreatic cancer patients are well tolerated. Of the 16 patients in the experiment, only 1 case (6%) developed vaccine-related hypertension and fever symptoms, with no adverse reactions [13].

The mRNA-5671 developed by Moderna shows anti-tumor activity by wrapping the mRNA cancer vaccine with lipid nanoparticles. After the ß vaccine is inoculated, the KRAS targeted peptide generated by mRNA is absorbed and translated by antigen presenting cells (APC). Antigens are presented to the immune system through the major histocompatibility complex (MHC) molecules on the surface of APC. Finally, cytotoxic T lymphocytes (CTL) and memory T cells are induced to specifically kill KRAS mutated tumor cells[14].

5. Difficulties and prospects of mRNA tumor immunotherapy

The potential of mRNA cancer vaccine from early research and development to clinical development is highlighted, and the technical obstacles it faces are also obvious. The difficulties and key technical points of mRNA cancer immunoassay are synthetic modification, such as to improve the stability of mRNA molecules and prevent degradation and delivery system, for example, to improve the efficiency of entering human cells and generate antigens to stimulate human immune response.

6. Conclusion

Human beings have made a series of breakthroughs in the research and development of mRNA cancer vaccines and drugs, bringing new prospects for human cancer treatment. Among the immune studies of mRNA, cancer immunotherapy with mRNA is the most widely studied[15]. Under the leadership of international leading pharmaceutical enterprises, domestic enterprises have also gradually increased their research and development efforts, but there is still a certain gap with foreign countries. With a series of advances in clinical stage, mRNA cancer immunotherapy has also brought more challenges to human beings.

References

[1] Farkona, S., Diamandis, E. P., and Blasutig, I. M. Cancer immunotherapy: the beginning of the end of cancer?. BMC Med. (2016)14:73.

[2] Lei Mia, Yu Zhang and Leaf Huang. mRNA vaccine for cancer immunotherapy. Open Access. (2021) 20:41.

[3] Granot, Y., and Peer, D. Delivering the right message: challenges and opportunities in lipid nanoparticles-mediated modified mRNA therapeutics-An innate immune system standpoint. Semin. Immunol. (2017)34:68-77.

[4] Wadhwa, A., Aljabbari, A., Lokras, A., Foged, C., and Thakur, A. Opportunities and challenges in the delivery of mRNA-based vaccines. Pharmaceutics. (2020).12:102.

[5] Kranz, L. M., Diken, M., Haas, H., Kreiter, S., Loquai, C., Reuter, K. C., et al. Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy.Nature. (2016)534:396-401.

[6] Pardi, N., Secreto, A. J., Shan, X., Debonera, F., Glover, J., Yi, Y., et al. Administration of nucleoside-modified mRNA encoding broadly neutralizing antibody protects humanized mice from HIV-1 challenge. Nat. Commun. (2017)8:14630.

[7] Elizabeth I., Anna M., Alexander K., Marc K. Jenkins. Detection of Dendritic Cell Antigen Presentation to CD4+ T Cells. J Exp Med. (1997)185: 2133-2141.

[8] Alexandra E. G., Stephen B., Jaya S., Courtnee A. C., Victoire C., Dongwook C. C.. A Linear Plasmid for Generating mRNA IVT Templates With Extended Encoded Poly(A) Sequences. Molecular Therapy—Nucleic Acids (2016) 16: 2162-2531.

[9] Jan D. Beck et al, mRNA therapeutics in cancer immunotherapy, Molecular Cancer (2021) 20:69

[10] Sasso, J. M. , Ambrose, B., Tenchov, R., Datta, R. S. , Basel, M. T. , & Delong, R. K. , et al.. The progress and promise of RNA medicine-an arsenal of targeted treatments. (2022)26:254-263.

[11] Boczkowski P. Review of Carl Mitcham's Thinking through technology: The path between engineering and philosophy: Science, Technology, & Human Values. (1996)17:31-37.

[12] Burris HA. A phase I multicenter study to assess the safety, tolerability, and immunogenicity of mRNA-4157 alone in patients with resected solid tumors and in combination with

pembrolizumab in patients with unresectable solid tumors; (2019)34:803-806.
[13] Ann J. B., Allen Y. J., Peng Z., Richard W. and Daniel G. A.. The clinical progress of mRNA vaccines and immunotherapies. Nature Biotechnology. (2022)3:840-854.
[14] Barbier, A.J., Jiang, A.Y., Zhang, P. et al. The clinical progress of mRNA vaccines and immunotherapies. Nat Biotechnol (2022)40:840-854.
[15]. Grimmett E., Al-Share B., Alkassab M., Zhou R., Desai A, Rahim M., Woldie I.. Cancer vaccines: past, present and future; a review article. Discov Oncol. (2022)13:31.