The Potential and Challenges of Circular RNA Vaccines: From Mechanism to Clinical Use

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Keywords: Circular RNA Vaccine, Stability, Immunotherapy.

Abstract: Circular RNA (circRNA) is a stable, covalently closed RNA that regulates gene expression by sponging microRNAs and can sometimes translate into proteins. This makes circRNA a promising platform for vaccines against infectious diseases and cancer immunotherapy. Compared to mRNA vaccines, circRNA vaccines offer longer half-lives, enhanced stability, room-temperature storage, and reduced immunogenicity, minimizing adverse reactions and logistical challenges. However, key challenges include inefficient delivery, high production costs, and uncertain long-term safety. Ongoing research focuses on optimizing circRNA design, improving delivery methods (e.g., lipid nanoparticles), and developing cost-effective production techniques. This review discusses circRNA's functions, its vaccine potential, and the obstacles to its clinical application in infectious disease prevention and cancer therapy.

1 INTRODUCTION

Circular RNA (circRNA) is a category of RNA with a covalently closed structure. Without a 5' end or a 3' end, circular RNAs have a higher stability than linear RNAs. As for the functions, circRNA can regulate many reactions in vivo. In terms of the mechanisms functions in vivo. The majority of circRNA is formed through a unique process called back-splicing. After that, circRNA can bind to miRNAs, acting as RNA sponge and regulate gene expression (Pan-da.2018). In addition, some circRNA is able to be translated into proteins, and induce immune response (Zhou et al. 2023). Therefore, circRNA have high potential in vaccine development. At pre-sent, circRNA vaccines against SARS-CoV-2 and monkeypox virus are already under development.

Compared to traditional mRNA vaccines, circRNA vaccines have multiple distinctive ad-vantages. Due to its closed-loop structure, circRNA is less prone to degradation, providing a longer half-life and sustained antigen presentation (Bu et al.2025). This extended exposure enhances immune response durability. In addition, traditional mRNA vaccines need to be stored in an ultra-cold and sterile environment, while circRNA vaccines have high stability and can be stored at room temperature or low temperatures, which is easy to transport. What's more, the low immunogenicity of circRNA allows it to alleviate the side effects of immune response, such as inflammatory response, thus expanding the range of applicable people. The purpose of this article is to review the latest progress of circRNA in vaccine development, and to explore its unique biological characteristics, immune mechanism, and application advantages. At the same time, this article will also evaluate the current challenges faced by circRNA vaccines, and by summarizing the existing research, this article will provide theoretical guidance for the future development of circRNA vaccines, and look forward to their application prospects in the field of immunotherapy.

2 circRNA AND ITS BIOLOGICAL MECHANISM

2.1 Biosynthesis of circRNA

Most circRNAs in vivo are formed by back-splicing. For circRNAs which have exons, if there are complementary sequences or repeated Alu sequences within the two introns flanking the exons, the complementary parts will come close to each other

Song, Y. and Liu, S.
The Potential and Challenges of Circular RNA Vaccines: From Mechanism to Clinical Use.
DOI: 10.5220/0014488100004933

In *Proceedings of the 1st International Conference on Biomedical Engineering and Food Science (BEFS 2025)*, pages 335-339
ISBN: 978-989-758-789-4

due to base-pairing, thus trigger back-splicing, forming a new circular pattern with the exons and the remaining parts of the flanking introns involved. (Hwang et al.2024) Additionally, RNA binding proteins (RBPs) can regulate the back-splicing. They can either facilitate the biosynthesis of exonic RNAs (EcircR-NAs) and exonic-intrinsic RNAs (ElciRNAs), or inhibit the splicing of circRNAs. The unique process of back-splicing gives circRNAs its special stability and functions.

2.2 Biological Function of Circ RNA

CircRNAs play a crucial role in biological processes. One key function is their "sponging" effect, where circRNAs bind to miRNAs, preventing them from interacting with target mRNAs and inhibiting miRNA-mediated gene suppression. In other words, circRNAs counteract RNA inhibition. Depending on the number of miRNAs bound to circRNAs, they can have a bidirectional effect on gene expression. A higher number of bound miRNAs suppresses mRNA degradation, leading to increased gene expression. Conversely, a lower number of bound miRNAs can reduce gene expression (Pamudurti et al., 2020). Additionally, circRNAs containing exons can be translated into proteins. These proteins and peptides can act as antigens, triggering immune responses. As a result, synthetically engineered circRNAs can serve as vaccines, providing immune protection and disease prevention.

2.3 Mechanism of circRNA Vaccine

The circRNA undergoes three main processes in the cell when it functions: endosome escape, antigen encoding, and immune initiation. In APCs, lipid nanoparticles (LNPs) containing circRNA vaccines form endosomes which are membrane-encapsulated vesicle structures in the cytoplasm. Then the endosomes release the circRNA vaccine, which is called endosome escape. In the next step, the circRNA encoding sequence is translated by the ribosome into an antigenic protein or peptide. Endogenous antigens are degraded into peptides by proteases and presented by MHC I molecules, activating cytotoxic T cells which are CD8+ T cells. In addition to cellular immunity, circRNA-induced humoral immunity is also important. Endogenous antigens in APCs can be secreted and presented by MHC II molecules to helper T cells, helper T cells that are CD4+ T cells, which further stimulate B cells to produce neutralizing antibodies (Dun et al., 2023).

3 THE POTENTIAL OF RNA IN VACCINE DEVELOPMENT

At present, the application of circRNA in vaccine research and development has gradually at-tracted attention, especially in the field of viral vaccines and cancer immunotherapy. In terms of viral vaccine research and development, circRNA vaccines have shown potential in the preven-tion of novel coronavirus, Mpox virus, Rabies virus and other viruses. In terms of cancer immu-notherapy, in cancers such as colorectal cancer, gastric carcinoma, and liver cancer, the expres-sion changes of circRNA and the relationship between these expression changes and immune response have been confirmed. Based on these differences, we can mobilize immune cells to re-spond to cancer cells by controlling circRNA expression, and then develop immunotherapies against these cancers (Wan et al., 2024).

There are currently circRNA vaccines that are being studied against the novel coronavirus. The research team synthesized circRNA using a linear template containing RBD coding sequences using in vitro transcription. Cyclization was performed using a group I intron method and a T4 RNA ligase-based approach. Experiments in mice have shown that circRNA vaccines can significantly reduce the infection of the SARS-CoV-2 beta variant, reduce the viral load in the lungs, and there is no obvious disease exacerbation (Qu et al., 2022). Through experiments in animal models, the research team found that circRNA vaccines performed well in preventing tumor growth and inhibiting tumor growth. Compared with linear mRNA vaccines, circRNA vaccines induce a higher proportion of tumor-specific T cells and have stronger immune activa-tion capabilities (Yu et al., 2025).

CircRNA vaccines also have great potential for the treatment of cancer. The research team has experimentally found that circRAPGEF5 and circMYH9 can translate distinctive peptides and activate CD8+ T cells. The research team detected the presence of circMYH9 in blood samples from colorectal cancer patients, suggesting that circRNA can be used as a biomarker in liquid biopsy for early detection of cancer and vaccine development, which is expected to improve treatment effect (Ren et al., 2024).

4 ADVANTAGES OF circRNA VACCINES

4.1 Stability

Without a traditional 5'end or 3' end, circRNAs are immune to degradation led by RNase. At the same time, CircRNAs have high stability due to the covalently closed structure. As a result, compared with mRNA vaccines, circRNAs have longer half-life *in vivo*, which means that circRNAs vaccines can be expressed at a longer time and be translated into more antigen proteins than mRNA vaccines. Thus, circRNA vaccines can induce immune response more effectively. Moreover, many traditional mRNA vaccines can only be stored and transported in highly restricted environment such as refrigeration, while circRNA vaccines can still maintain its functions in room temperature, which reduces the transportation and storage cost.

4.2 High Translation Efficiency

circRNA has a unique translation mechanism, leading to high translation efficiency. The translation initiation of circRNA is dependent on Internal Ribosome Entry Site (IRES) or m6A modification instead of conventional 5' cap, increasing the translation efficiency. In addition, when one round of translation is over, the ribosome can directly pass over the circRNA even if after reaching a stop codon and start the next round of translation right away (Xie et al., 2023). This kind of translation is called rolling circle translation, which may seem slow but actually effective. As a result, more stable and efficient antigen production can be achieved, enabling stronger immune protection.

4.3 Immunogenicity and Self-Adjuvanted Vaccination

CircRNA itself has immunogenicity and has the ability to activate immune cells like natural killer cells and antigen presenting cells, and it can promote the presentation of antigen translated by itself. Subsequently, circRNAs are able to enhance the immune response against the antigen produced by themselves and be made into self-adjuvanted vaccines. Compared with mRNA vaccines, circRNA vaccines induces higher antibody production and effective immune protection (Das et al., 2024).

5 PREPARATION PROCESS

5.1 Sequence Adjustment and Modification

To use circRNA in vaccine development, the first step is to synthesize linear precursor RNA. Since there is no 5' end, circRNA cannot initiate translation in a conventional 5' end-determined manner. Therefore, sequence optimization of circRNAs is required for successful initiation of translation. The first manner is the addition of an untranslated region (UTR) containing an RNA-binding protein (RBP) motifs upstream or downstream of the IRES-ORF gene cassette of the linear RNA. IRES can recruit ribosomes through IRES-transacting factors (ITAFs) to initiate translation without the involvement of traditional translation initiation factors. The most efficient IRES currently known is EV-A IRES (Yu et al., 2025). Another approach is to introduce m6A modification upstream of circRNA and use it to mediate translation initiation. This modification may reduce the immunogenicity of circRNA (Niu et al., 2023).

5.2 Linear RNA Precursor for Circularization

Next, the translated precursor RNA needs to be cyclized to become circRNA and be used as a vaccine. At present, there are three cyclization methods for circRNA: chemical synthesis, T4 ligase method and ribozyme ligation. The chemical synthesis method uses chemicals to induce the formation of phosphodiester bonds between the 5' end and 3' end of linear RNA to form circRNA. The T4 ligase method involves the use of T4 DNA ligase, T4 RNA ligase I, and T4 RNA ligase II. These ligases can directly form phosphodiester bonds between the 5' end phosphate group and the 3' end hydroxyl group of linear RNA through nucleotide transfer reactions, thus forming circRNA. Ribozymes are bioactive RNA molecules with catalytic functions. There are several ribozyme-based methods, the most prominent of which is the arrangement of introns and exons (PIE) methods. The group I intron self-splicing system is more commonly used than the group II intron self-splicing system. In this method, two continuous transesterification reactions are achieved with the help of type I intron self-splicing system or type II intron self-splicing system to form circRNA (Niu et al., 2023).

5.3 Vaccine Delivery System

The circRNA vaccines need to be delivered into cells to function. The most common is that using lipid nanoparticles (LNP) delivery systems, which are a new generation of liposomes with high biocompatibility and biodegradability. LNP delivery system can improve the stability and permeability of circRNA vaccines, which is a typical and widely used delivery system with significant advantages. In addition to LNP delivery system, using viral vectors, electroporation techniques and exosomes can also deliver circRNA vaccines. Viral vectors are rarely used for circRNA delivery. When using viral vector delivery, the viral vector integrates DNA as an intermediate into the host genome, then transcribes it into RNA, and then cyclize it into circRNA in cells. Electroporation technology uses short high-voltage pulses to form temporary holes in the cell membrane to allow RNA molecules to enter the cell. Electroporation is a versatile and efficient method for non-viral RNA delivery. Exosomes are natural lipid bilayer vesicles with good biocompatibility and low immunogenicity (Cai et al., 2024).

6 CHALLENGES AND IMPROVEMENT STRATEGIES FOR circRNA VACCINES

At present, the industrial production of circRNA vaccines is not yet mature, and its production process mainly includes three stages: linear RNA molecules are synthesized through in vitro transcription using plasmids as templates, RNA circularization and vaccine encapsulation. High-purity plasmids are critical to vaccine quality, and the industrial production of plasmids goes through processes such as fermentation, bacterial harvest, lysis, clarification, ultrafiltration and concentration, and chromatographic purification. During chromatographic purification, RNA, trace impurities, and endomycin can be removed effectively by adding different types of filler, thereby obtaining high-purity plasmids (Bai et al., 2023). In the process of RNA synthesis, intron self-splicing is more suitable for linear RNA synthesis with larger molecular weight than other methods. The purity of circRNA directly affects its low immunogenicity and protein expression levels. The main purification methods are RNase R treatment, high-performance liquid chromatography (HPLC) and electrophoresis. These methods are effective in separating linear RNA from circRNA (Zhang et al., 2024). In terms of delivery systems, LNP delivery system is currently widely recognized and applied RNA vaccine delivery methods, but they have poor heat tolerance, resulting in extremely low temperatures for vaccine transportation and storage. Meanwhile, using LNP may cause side effects such as inflammation. As an emerging technology, exosome delivery has gained more and more attention, and its advantage is that it can effectively reduce side effects, but large-scale industrial production still faces great challenges and needs to be further studied and optimized (Zhao et al., 2022).

Additionally, as a kind of vaccine in clinical examination stage, there are still uncertainty about the immune response and side effects. Humans generate endogenous circRNAs, and those endogenous circRNAs participate in the regulation of many essential chemical effects. it is still unknown whether exogenous circRNAs will interfere the normal regulation effects. As a result, to be sure about the side effects, more clinical tests are needed. At the same time, up to now, the preparation process and generation equipment are not mature enough, so the generation procedure is complicated and the scale of production is low, not able to satisfy the massive requirements in clinic use. Besides, due to the complex equipment and high expense, the number of patients who may use the product might be low. In order to make breakthroughs, more equipment that can be used in massive production of circRNA vaccines needs to be developed to reduce manufacture costs and difficulty.

7 CONCLUSION

With its unique covalent closed-loop structure, circRNA vaccines demonstrate the advantages of long lifespan, high stability, low immunogenicity and translatability. However, its development still faces many challenges, including improving the cyclization efficiency of linear RNA pre-cursors, improving purification methods to obtain high-purity circRNA and establishing an efficient and safe delivery system. The self-adjuvanting effect of circRNA vaccines enhances the intensity and duration of the immune response, which is crucial for improving vaccine effectiveness. In animal models, circRNA vaccines have been proven to activate cellular immune response effectively, especially CD8+ T cell-mediated immune response, thus circRNA vaccines

have good anti-tumor effects. Moreover, some circRNA have been found to be potential tumor biomarkers, but a large number of research is required to validate that in aspect of clinical applications. In summary, circRNA vaccines have broad development prospect in the field of infectious disease prevention and immunotherapy for tumor, and may be used as an effective treatment for major infectious diseases or common viral diseases in the future, and may also be developed into therapeutic tumor vaccines.

AUTHORS CONTRIBUTION

All the authors contributed equally and their names were listed in alphabetical order.

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