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Application of Synthetic Biology in SARS-CoV-2 Vaccine Development

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Abstract. The novel coronavirus SARS-CoV-2 has been spreading at an extraordinary rate since it first appeared in late 2019, causing a large number of infections and deaths. The COVID-19 pandemic can be successfully controlled most effectively with vaccines, and efforts are being made globally to create new vaccines. Synthetic biology is a design-oriented field that focuses on creating novel biological functions by discovering, analyzing, and repurposing biological components. Many SARS-CoV-2 vaccines have been developed using synthetic biology, and the recent approval of a few more vaccines is evidence of the field's continuous growth. This review discusses the use of synthetic biology technology in the creation of several COVID-19 vaccines, concentrating on how to produce vaccines that are safer and more effective through genome optimization and delivery mechanism optimization. Finally, this article highlights some existing problems of synthetic biology technology and provides an outlook on the future development of application this technology in COVID-19 vaccines.

Keywords: SARS-CoV-2, COVID-19 Vaccine, Synthetic Biology

1. Introduction

Since the end of 2019, a new variant of the severe acute respiratory syndrome (SARS-CoV-2) virus has been responsible for COVID-19, an infectious disease that has resulted in a significant number of morbidities and fatalities globally [1]. SARS-CoV-2 is a single-stranded positive-strand RNA beta coronavirus with a genomic size of 30 kb. Its 14 ORFs are made up of the structural components nuclear membrane protein (N), spike protein (S), membrane protein (M), and surface protein (E). The N protein is responsible for the transcription and replication of mRNA and the budding of the virus, the M protein is responsible for the formation of the viral envelope, and the E protein is the main virulence factor of the virus and contributes to the secretion of inflammatory mediators. The S protein, which has two subunits called S1 and S2, has received the most in-depth research. For infection, S1 contains the RBD that can attach to the ACE2 on the cell surface, and S2 is in charge of fusing the virus with the host cell membrane so that the genetic material of the virus can enter the cell. Several antibodies, including anti-S protein, anti-RBD, and anti-N protein, are present in the serum of COVID-19 patients who have recovered, although the anti-S protein and anti-RBD antibodies primarily serve antiviral functions by inhibiting the S protein from binding to ACE2. Therefore, most current vaccines target the S protein and the RBD region of the S1 subunit.

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Since there is currently no medication that can entirely treat the illness, many measures are tried to stop its spread, the most successful of which is to boost herd immunity. The sole ethical strategy for establishing herd immunity is thought to be the development of vaccines that provide long-lasting immunity [2]. As of July 2022, 169 vaccines have entered clinical research worldwide, including inactivated virus vaccines, live attenuated vaccines, nucleic acid vaccines (DNA and RNA vaccines), viral vector vaccines (replicating and non-replicating vaccines), protein subunit vaccines, and virus-like particle vaccines [3]. Nevertheless, as the virus has spread, more and more mutant strains have emerged, which has made it extremely difficult to avoid epidemics. Therefore, it is essential to create vaccines that are safer and more effective.

As an emerging technology in the 21st century, synthetic biology aims to use engineering concepts to rationally design, synthesize and reconstruct existing genetic elements or construct new genetic parts to construct complex and biologically meaningful systems at different levels to perform various functions. Synthetic gene circuits contain basic signal-response regulatory elements that can be activated by specific exogenous stimuli or endogenous metabolites to regulate subsequent gene expression. Compared with traditional genetic engineering, synthetic biology has the characteristics of rapid synthesis, accurate gene construction, and easy acquisition of mutations, which provides a new research idea of "building knowledge" for life sciences. Therefore, the concepts and methods of synthetic biology are widely used in biomedicine, injecting new ideas and strategies into modern medicine.

Synthetic biology techniques are also being utilized in the research and development of SARS-COV-2 vaccines to get perfect output through precise input which increases the protein production of nucleic acid during the vaccination process to improve the speed and effectiveness of vaccine development. In the context of the worldwide new crown pandemic, people need to effectively use biological data and bioengineering technology. At the same time, the research method of synthetic biology can better build a vaccine development platform for researchers, exercise the technical ability of emergency vaccine development, and respond to the possible threats to human health in the future to the greatest extent. This paper reviews the use of synthetic biology in the research and development of SARS-CoV-2 vaccines, focuses on the most recent advances in the development and production of various types of vaccines using synthetic biology, expounds on the significance of this technology in the field of SARS-CoV-2 vaccines, and summarizes and forecasts the future development direction of this technology in the field of SARS-CoV-2 vaccines.

1.1. Live attenuated vaccine

Live attenuated vaccine weakens the virulence of the virus after a series of treatments, but it still has immunogenicity. After treatment, the virus will not cause disease but can reproduce in the human body, thus causing an immune response and playing a protective role. It has the advantages of strong immune effect, long immune duration, low cost, and so on. However, with the increasingly serious epidemic situation of COVID-19, there is an urgent need for vaccines, especially boosters, and some shortcomings of live attenuated vaccines have also been exposed. As live attenuated vaccines retain a certain residual virulence, serious diseases may be induced in some individuals (such as immunodeficient). And due to a variety of reasons (such as genetic modification), the vaccine may have a "virulence atavism". In addition, the conventional method of attenuating the virulence of the virus takes too long, and it is easier to produce inactivated virus vaccine, but this also leads to poor immune effects [4]. Therefore, how to improve the efficiency of detoxification has become one of the urgent problems to be solved.

Using the method of synthetic biology, the virus genome can be redesigned by synonymous mutation, and the degeneracy of codons can be used to change the expression content of viral protein in human cells, to produce attenuated virus quickly and reliably. Ying Wang et al. used the CDX-005 strain of SARS-CoV-2 to introduce 283 synonymous point mutations to re-encode the genome of S protein, and then detected the viral load of the vaccine after injection into hamsters, and found that the viral load was reduced by more than 50,000 times on average, which proved the effectiveness of the

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vaccine [5]. To solve the phenomenon of "virulence atavism" caused by virus mutation after vaccination, reverse genetics has been used in SARS-CoV-2 vaccine development, such as the CPER method, which combines a variety of mutations to keep the virus in a state of virulence decline and can be used as a safe live attenuated vaccine [6].

1.2. Genetic factor

In 1997, SNCA (PARK1, PARK4) was the first gene that were found to be PD causative gene. Some people also named it as α-synuclein genes since mutations in the α-synuclein genes Ala53Thr and Ala39Pro lead to abnormal deposition of α-synuclein and eventually the formation of LBs. Overexpression of α-synuclein in mice or Drosophila produces the classic symptoms of PD. In addition, it has been shown that SNCA (PARK1, PARK4) promoter polymorphism variants increase the risk of sporadic PD, and that multiplication of SNCA wild-type motifs is also quantitatively associated with PD susceptibility. For example, additional copies of SNCA lead to earlier age of onset and more severe symptoms such as dementia [3-4]. Although mutations in the α-synuclein gene are only found in a small proportion of patients with familial PD, the fact that the protein expressed in this gene is a major component of LBs suggests that it plays an important role in causing the a-Syn pathogenesis of PD.

1.2.1. DNA Vaccine. A DNA vaccine is favored because of its strong stability and ability to reduce non-specific inflammation, but it also faces many problems, such as poor immunogenicity when it is injected into human cells and the risk that DNA fragments may be integrated into human genes. There are still some aspects of DNA vaccines that can be improved. Through a series of design optimization to improve its immunogenicity, it has become an important choice of vaccine under the epidemic situation of COVID-19.

Codon optimization is a common method. Due to the codon bias, some rare codons in the RNA transcribed by the DNA vaccine cannot be fully recognized by the host cell tRNA, which leads to low translation efficiency and poor vaccine immunogenicity. Therefore, codons can be screened and optimized before gene synthesis and cloning. While optimizing the antigen gene, we should also screen and optimize the vector, including the strong promoter and terminator. We should also consider signal peptide sequence selection and controlling the level and position of protein production to improve the immunogenicity of the DNA vaccine. TrevorR.F.Smith et al. used sequence alignment to screen the trimer sequence of S protein of SARS-CoV-2 and inserted an IgE sequence to the N-terminal. A computer genetic optimization approach was used to generate the highly optimized DNA sequence encoding the IgE fragment of S protein. The vaccine can completely express the same trimer structure in mice and guinea pigs. This trimer structure can greatly promote the activation of humoral and cellular immunity [7]. Other methods, such as the design of promoter, optimization of adjuvant and delivery system, optimization of delivery systems (such as electroporation), epigenetic and RNAi techniques, and genomics methods, are helpful to the design and optimization of DNA vaccines [8].

1.2.2. RNA Vaccine. RNA vaccine is composed of mRNA molecules that encode antigens, which do not need to be transcribed. Antigens can be produced after delivery to human cells through translation, so there is no need to worry about genome integration compared with DNA vaccines. At the same time, the RNA vaccine is easy to design and manufacture. But accordingly, one of the main challenges faced by RNA vaccine is how to transfer the complete transcript across the lipid bilayer into the cell translation and produce an antigen, because RNA is easily degraded by nuclease in this process. In addition, RNA degradation can easily lead to inflammation and cytotoxicity, which will reduce the immunogenicity of the vaccine.

Scientists use synthetic biology methods to optimize the delivery pathway of RNA vaccines, improve the stability of RNA in cells and reduce the inflammatory response. For example, lipid nanoparticles (NPs) are utilized to concentrate, preserve, and improve RNA delivery in cells, and by adding modified nucleosides (especially modified uridines), optimizing the coding sequence, and

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purifying IVTmRNA by HPLC technology to remove double strands RNA contaminants and other methods improve the stability of RNA in cells, reduce cytotoxicity, increase protein production, etc. [9]. These technologies are also inextricably linked to the study and development of an mRNA COVID-19 vaccine. The mRNA-1273 vaccine developed by Moderna is modified by nucleoside to encode a full-length S protein with a transmembrane anchor that is stable in the pre-fusion form of two prolines. The vaccine produces an effective antibody neutralization reaction [10]. In phase 3 clinical trials of the vaccine, the point estimate of the efficacy of the vaccine was 94.1%, indicating that the mRNA-1273 vaccine has a strong protective effect on serious diseases. PaulF.McKay et al. proposed a self-amplifying RNA (saRNA), which is developed from Alphavirus and can encode the target gene and transmit S protein through NPs, so that RNA can replicate when delivered to the cytoplasm and increase the expression of the protein. As a result, it can reduce the time from gene synthesis to assembly and release of vaccines and improve production efficiency [11].

1.2.3. Viral vector vaccine. Viral vector vaccine transduces antigen genes into harmless microorganisms to induce an immune response. Due to viruses' intrinsic propensity to infect host cells, viral vector vaccines have a high gene transduction potential. Adjuvants are not required for these vaccines since viral components boost the innate immune system [12]. Viral vector vaccines are divided into replicative and non-replicating types, replicative viral vectors can produce infectious offspring, so they have a high level of immunogenicity, but there is a certain risk of virulence atavism; non-replicating viral vectors cannot produce infectious offspring because of the removal of genetic material, but the immunogenicity is reduced accordingly, and a larger dose is needed to complete immunization.

Synthetic biology is committed to optimizing target genes and viral vectors by synthetic methods so that they have high immunogenicity as well as high safety. Synthetic gene circuits that are programmable and modular can be inserted into adenoviral vectors for immunotherapy. The viral vector vaccine AZD1222 created by Oxford University and AstraZeneca uses attenuated gorilla adenovirus ChAdOx1 to encode wild-type S protein of SARS-CoV-2 and human 5'- terminal tissue plasminogen activator (tPA). TPA can enhance the immune prototype and optimize the S protein codon of SARS-CoV-2 to be cloned into shuttle plasmids and expressed in human cell lines. At the same time, cytomegalovirus (CMV) promoter, tetracycline operator (TetO) site and bovine growth hormone (BGH) polyA signal sequence were added on both sides to enhance the immunogenicity of the vaccine [13]. Kangxinuo has developed a candidate vaccine Adn5-CoV using human adenovirus serotype 5 vectors (Ad5). The non-replicating vaccine incorporates an improved SARS-CoV-2 full-length S protein sequence and tpA leader sequence that has demonstrated good immunogenicity in clinical studies, particularly in critically ill patients [12].

1.2.4. Recombinant protein vaccine. Recombinant protein vaccine has the advantages of high safety and good stability by extracting immune active fragments of pathogens and stimulating the body to produce antibodies. S protein is responsible for binding host cell receptors and facilitating membrane fusion. Based on the S protein, a SARS-CoV-2 vaccine can efficiently promote antibody formation and virus neutralisation. As a result, the S protein is a promising target for vaccine development. However, adjuvants are frequently required, and the immunogenicity is modest. Therefore, it is necessary to select appropriate adjuvants and synthesize some fragments to enhance the immunogenicity of the vaccine.

GlaxoSmithKline (GSK) has produced a recombinant SARS-CoV-2 protein vaccine that incorporates S protein immunogens and oil-in-water emulsion adjuvants that elicit strong levels of anti-SARS-CoV-2 IgG2a or IgG2b antibody responses in animals. The S-Trimer subunit vaccine was rapidly expressed in mammalian cell expression system by Trimer-Tag technology. This trimer tag can promote the production of new covalent trimer fusion protein and enhance the targeting and immunogenicity of the vaccine [14]. The RBD region is also a key area of vaccine development, which contains a variety of conformational-dependent epitopes and can induce high titers of

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neutralizing antibodies. ZezhongLiu et al. spliced the RBD fragment with the Fc fragment of human IgG to construct the recombinant protein vaccine. It was discovered that the recombinant protein vaccine could induce a high titer of RBD-specific antibody in mice, indicating that the recombinant protein vaccine has great potential and can be developed further into an efficient broad-spectrum vaccine [15]. Methods of immunoinformatics and comparative genomics can help predict peptide vaccines based on T cell epitopes to cope with a large number of mutations produced by the SARS-CoV-2 virus.

2. Conclusion

Vaccine development and clinical application are critical. This is the primary solution to the problem. In the unprecedented new crown epidemic sweeping the world, scientists all across the world are working hard to create a safe and effective vaccine to withstand and end the pandemic. The scientific progress led by the previous pandemics of SARS and MERS has accelerated our understanding of SARS-CoV-2 vaccine development, but traditional technical means still suffer from slow development and low production efficiency. Synthetic biology, as a new multidisciplinary field of biology developed in recent years, can use the modularization of existing biological components to create new systems, and at the same time optimize existing systems to achieve the purpose of rapid development of new vaccines. Current research has demonstrated the feasibility of synthetic biology approaches in the development of various types of vaccines, including the use of bioinformatics and computational biology to screen specific antigens with high immunogenic potential, optimization of genomes, and improved nucleic acid Vaccine delivery methods, etc., to rapidly manufacture targeted, effective and low-side effects vaccines to effectively deal with various mutant strains of SARS-CoV-2.

Safety and efficacy are primary qualities of vaccines, so further specifications and tests are still needed for the development of current vaccines. On the one hand, synthetic biology technology itself has certain biosafety risks. Since synthetic biology will introduce biological and non-biological "components", it has certain complexity and uncertainty, and the resulting final product may have the hidden danger of higher pathogenicity and infectious biosafety risk. Strengthening the regulation and regulation of the use of synthetic biology approaches to study SARS-CoV-2 vaccines is critical. On the other hand, synthetic biology technology is still not mature enough, and it is still in its embryonic stage in the field of vaccine research and development. Many vaccines are still in the experimental stage of animal models and have not yet been used in clinical trials. For this reason, scientists must do a good job in animal models. Fully evaluate and conduct experiments on more volunteers of different ages and health statuses in follow-up clinical trials, and conduct the long-term evaluation of adverse events after vaccination to ensure the maximum safety of the vaccine. Furthermore, the selection of vaccine epitopes, the selection of vaccine adjuvants, and the evaluation of vaccine effects cannot be ignored.

As an emerging discipline in the post-genome era, synthetic biology has shown great vitality and broad prospects, and its feasibility and importance in many industries such as vaccine research and development are obvious to all. Accelerating the application of synthetic biology technology will definitely promote the development of the biomedical field. In the near future, it is hopeful to use synthetic biology technology to develop safer and more effective vaccines to combat the COVID-19 epidemic.

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