Journal of Trends in Life Sciences
ISSN: 2960-0200

APPLICATION OF REVERSE GENETICS IN THE DEVELOPMENT OF CORONAVIRUS VACCINE

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Abstract: In recent years, more and more coronaviruses (CoV) have crossed the species barrier and spread from animals to humans, causing many serious public health events and causing huge losses to the global economy. Vaccination is currently the most effective measure to prevent the CoV pandemic, and a safe, efficient, and fast vaccine platform is the basis for vaccine research and development. With the increasing maturity of reverse genetics technology, it is also increasingly used in the development of CoV vaccines. This article reviews several reverse genetics techniques currently used in CoV research and their possible applications in the development of CoV vaccines.
Keywords: Coronavirus; Reverse genetics; Vaccine

1 INTRODUCTION

Coronavirus (coronaviruses, CoV) is a major threat to human life and health, and the pandemics caused by it have broken out many times. , SARS-CoV-2) as an example, because of its extremely high infectivity, pathogenicity and variability, it has dealt a heavy blow to the economy and public health security of countries around the world. As of May 10, 2023, the world's SARS-CoV-2 has killed nearly 7 million people, while more than 19 variants of concern are spreading globally, including α, β, γ, and o [1].

At present, the most effective way to deal with the SARS-CoV-2 epidemic is to establish herd immunity through vaccination, block or slow down the spread of the virus in the population, reduce the morbidity rate, severe disease rate and fatality rate, and then end the virus epidemic. However, with the continuous emergence of SARS-CoV-2 variants and immune escape, the protective efficacy of existing marketed vaccines is facing great challenges. New vaccines that can target mutant strains are urgently needed to be developed. However, there are some problems in the existing vaccine research and development strategies. Taking inactivated vaccines as an example, although inactivated vaccines have the advantages of safety, efficacy, and strong immunogenicity, the production conditions of vaccines are relatively high, and it is difficult to obtain suitable candidate vaccine strains[2].

Aiming at some problems faced in the current SARS-CoV-2 vaccine development process, the reverse genetics system is a very potential tool. Compared with classical genetics, the reverse genetics system can produce mutant and recombinant viruses through genome modification , quickly, efficiently, and directionally study the biological functions and mechanisms of different regions or sites of the CoV genome, and obtain recombinant strains with specific traits. Understanding the pathogenic mechanism and invasion mechanism of the virus can help to study specific vaccination methods, so as to exert the protective effect of the vaccine more efficiently. In the study of the pathogenic mechanism of SARS-CoV-2, the experimenters used the reverse genetics system to replace the open reading frame (ORF) 7 of SARS-CoV-2 with GFP and GFP-nLuc reporter genes, Through recombinant virus infection, the susceptibility of the nasal cavity to SARS-CoV-2 was proved, and it was speculated that the nasal cavity is the initial site that mediates virus infection of the lungs [3], which means that intranasal administration may be a preventive method for SARS-CoV-2 2 more effective way of infection. In another study on feline infectious peritonitis virus (FIPV), the experimenters constructed a reverse genetics system based on serotype I FECV by replacing the spike protein. A recombinant virus that can cause persistent infection in cats has been rescued [4], which provides a powerful tool for the study of FIPV genotype conversion. This article presents a review of several reverse genetics techniques currently used in CoV research and their possible applications in the development of CoV vaccines.

2 COMMONLY USED REVERSE GENETICS SYSTEMS IN COV

CoV is an enveloped, positive-sense RNA virus belonging to the order Reticuloviridae, family CoV, and has the largest genome of any RNA virus known. Since the positive-strand RNA viral genome can directly initiate the translation of viral proteins and the transcription of viral negative-strand RNA, the virus life cycle can be initiated [5]. When the cDNA containing the full length of the viral genome is modified and positive-strand RNA is prepared and transfected into cells, complete and infectious virus particles can be obtained. Therefore, CoV is an important platform for reverse genetics research. The reverse genetics system can be used to make specific modifications to the CoV genome, so as to better study the biological functions of different genes of the virus, obtain various recombinant strains with specific traits, and provide more safety and security for the development of vaccines. Efficient way [6]. At present, the reverse genetics technologies applied to the CoV genome mainly include: targeted RNA recombination, in vitro ligation, bacterial artificial chromosome system (bacterial artificial chromosome, BAC), poxvirus vector and yeast artificial chromosome (yeast artificial chromosome, YAC) Transformation-associated recombination (TAR) technology. The following is a brief introduction to these commonly used technical routes. 1.1 Viral RNA-based reverse genetics system Targeted RNA recombination is the first reverse genetics system applied to CoV research[7], which mainly utilizes the expression of RNA polymerase in the process of RNA virus replication. A template transfer mechanism [8] that enables the manipulation of genes outside of the

CoV ORF1ab region in the absence of viral infectious full-length cDNA. It mainly includes two steps: first, construct a recombinant chimeric virus with a screening marker; second, construct a wild-type corresponding fragment of the screening gene and RNA of a fragment in the viral genome that needs to be operated, and transfer it to Infect the cells infected with the chimeric virus, and obtain the recombinant virus introducing the target mutation by means of negative selection. This protocol was first applied to the reverse genetic engineering of mouse hepatitis virus (MHV), and later applied to the full-length cDNA of porcine epidemic diarrhea virus (PEDV), FIPV and other CoVs. build [9-10]. However, when this scheme is applied, it needs to retain the replication ability of the virus itself, so it cannot operate on the virus replicase-related gene, namely ORF1ab, which greatly limits its application in the research of virus replication and toxicity . Some researchers used the reverse genetics system based on RNA recombination to replace the S protein gene of FIPV with the corresponding part of MHV, so that the constructed recombinant CoV could proliferate in new host cells, and it is a virulence agent for live attenuated vaccines and inactivated vaccines. This research and development provides a reference .

2.2 Reverse Genetics System Based on Full-length Viral CDNA

1.2.1 Reverse genetics system based on in vitro linearization

This scheme was first proposed by ALMAZAN et al. in the study of yellow fever virus. It mainly generates full-length viral cDNA by connecting viral genome cDNA fragments in vitro , and then use it as a template to transcribe and synthesize viral genome RNA. In application, the restriction endonuclease restriction site on the CoV genome is usually analyzed first, and primers are designed near the restriction endonuclease site to obtain a set of gene fragments covering the full length of the CoV genome. At the same time, at the front of the 5'-end gene fragment, add a restriction site for connecting the T7 RNA polymerase promoter, and add a restriction site for introducing a poly-A tail at the 3'-end. When constructing fragments, they are usually cloned into different plasmids first, so as to facilitate the large-scale amplification of the target fragments. After obtaining the target fragment, use restriction endonuclease to digest specific ends, and obtain a full-length cDNA containing the entire genome of CoV, T7 promoter and poly-A tail[8] through enzyme ligation, and use the whole The long cDNA is used as a template, and the viral genome RNA is transcribed and then transfected into a specific host cell to rescue and obtain the target virus strain. The subsequent construction of the recombinant virus only needs to modify the specific site of the corresponding gene segment. In addition, a study on MHV showed that if the mRNA expressing the N protein of the strain to be rescued is transfected with the full-length viral RNA transcribed in vitro, the replication and transcription ability of the viral RNA genome can be significantly enhanced, which is beneficial to Virus rescue. In recent years, with the development of circular polymerase extension reaction (CPER) technology, circular cDNA can be synthesized in vitro with a fragment of the CMV promoter sequence and a group of fragments containing the CoV genome, which is more rapid and efficient. Efficient and easy to achieve CoV rescue .

This scheme does not need to rely on the bacterial system to construct the full-length viral cDNA. Compared with the cDNA construction method relying on the bacterial system, it can effectively solve the instability and toxicity of the full-length viral cDNA in bacteria. At the same time, viral cDNA fragments can be manipulated and prepared on plasmids, which reduces the workload and difficulty to a certain extent. At present, it has been applied in the construction of infectious cDNA of coronaviruses such as SARS-CoV, Middle East respiratory syndrome (Mid-dle East respiratory syndrome coronavirus, MERS-CoV), PEDV and SARS-CoV-2. Taking SARS-CoV-2 as an example, in SARS-CoV-2

At the first time of the CoV-2 outbreak, WAND et al. quickly obtained the full-length cDNA of the virus genome by using the in vitro ligation strategy. After inserting reporter genes such as nLuc and mNeonGreen, as a platform for drug screening and vaccine evaluation, it has greatly promoted the development of SARS-CoV-2 antiviral drugs and vaccines.

1.2.2 BAC-based reverse genetics system

Compared with in vitro linearization-based reverse genetics technology, BAC-based reverse genetics technology is to connect the obtained DNA fragment containing the full-length CoV genome to DNA on the carrier. Due to the large CoV genome, it is difficult for the full-length cDNA of the virus to exist stably in the general plasmid, but PEN-ZES et al. overcome this problem in the process of constructing the full-length cDNA of TGEV in 2000. At the same time, by adding Escherichia coli F factor (fertility-factor), the copy number of BAC in Escherichia coli can be strictly controlled, so that during the proliferation of host cells, each cell only retains 1 to 2 copies of artificial chromosomes, It greatly guarantees the stability of the full-length cDNA of the viral genome and its sequence-related host cell toxicity. Its construction strategy is mainly as follows: First, several DNA fragments with homologous sequences are obtained through RT-PCR or chemical synthesis. The DNA fragment at the 5' end of the viral genome was ligated by PCR to add the homology arm sequence required for connection with the promoter and the vector plasmid, and the DNA fragment at the 3' end contained a poly-A tail and the homology arm sequence required for connection with ABC. Then, all DNA fragments are ligated to the selected ABCs in a specific order. Finally, under the action of T7 or CMV promoter, the corresponding recombinant virus can be obtained by post-transcription transfection (T7 promoter) or direct transfection (CMV promoter) cells in vitro.

The main advantage of this solution is that it can stably insert larger DNA fragments. At the same time, through the modification of F factor, unique cloning site, suitable promoter, resistance selection marker, etc., it can greatly optimize the construction, modification and screening of full-length viral cDNA And the process of virus rescue. After being constructed on the corresponding plasmid vector, a large number of vectors containing the full-length cDNA of the virus can be obtained, and subsequent gene modification can be carried out on the plasmid, which can speed up the construction process of the full-length cDNA of the recombinant virus. Using this scheme, the researchers carried out heterologous substitution of the S protein of PEDV, and the resulting recombinant virus has different neutralizing properties from the original PEDV, suggesting the possibility of constructing a recombinant adenovirus with heterologous S protein as an attenuated candidate vaccine strain.

1.2.3 Reverse genetics system based on poxvirus vector

The main feature of the reverse genetics technology based on poxvirus vector is that the poxvirus genome is selected as the vector carrying the full-length cDNA of the virus genome. Poxvirus vector is a universal vector for reverse genetics research. It can insert large fragments of foreign genes (26 000 ~ 31 000 bp) without affecting virus replication. At the same time, the genome of vaccinia virus is stable and infectious. It can be replicated efficiently in tissue culture, and it is easy to obtain strains with higher titers. When the program is applied, the genome sequence of the poxvirus should be analyzed first to find the restriction enzyme cutting site for the insertion of foreign cDNA. When the cDNA fragment containing the full-length CoV genome is inserted into the poxvirus genome, linearized and transfected into BHK cells, the corresponding virus particles can be rescued. This protocol was first applied to the construction of full-length cDNA of HCoV-229E, and was subsequently applied to CoVs such as infectious bronchi-tis virus (IBV), MHV, SARS-CoV, and FIPV. Using poxvirus vectors as the reverse genetics operating system of CoV, on the one hand, it can study the genetic characterization of CoV, laying a theoretical foundation for the development of CoV attenuated and inactivated vaccines; on the other hand, after removing the essential genes of CoV, the constructed Recombinant poxviruses can be used as candidate virus vector vaccines and have a very broad application prospect.

1.2.4 Reverse genetics system based on multi-plasmid vectors

The reverse genetics technology based on multi-plasmid vectors mainly solves the problem of difficulty in obtaining full-length cDNA and in vitro transcription due to the large genome of CoV in CoV reverse genetics research. relatively difficult problem. Its construction steps are mainly as follows: First, according to the characteristics of the CoV genome, it is divided into three or more main segments, and the corresponding DNA segments are obtained by reverse transcription or in vitro synthesis; secondly, the obtained segments are connected to different plasmids ; Finally, the corresponding virus particles can be obtained by co-transfecting all the plasmids containing different parts of the CoV genome into 293T cells.

The main advantage of this scheme is that the construction of the plasmid is relatively simple, and the target recombinant virus can be obtained quickly. And because the viral genome cDNA exists on different plasmids, the operation is relatively simple. However, due to the inability to produce RNA containing the full length of the viral genome, only empty shells of virus particles can be formed, and continuous passage cannot be performed. This protocol has now been successfully applied in SARS-CoV-2. In the study of the cell tropism of PEDV, the researchers found three sites on the S protein related to the Vero cell tropism of PEDV through the reverse genetics technology of multi-plasmid vectors, suggesting that the cell tropism of PEDV It may be related to the cleavage site of its S2 subunit or the structure of RBD, providing theoretical guidance for CoV Vero cell-adapted vaccine candidates.

1.2.5 Reverse genetics system for YAC-based TAR cloning

The size limitation and instability of viral sequences are major obstacles to cloning the full-length cDNA of the CoV genome into replicable vectors, although BAC-based strategies are somewhat These problems have been overcome, but the cytotoxicity of some viral sequences to bacteria still limits the construction of full-length cDNA of part of the CoV genome. Compared with bacteria, Sac-charomyces cerevisiae is less sensitive to cytotoxicity of viral sequences. In addition, through the TAR cloning technology, the operation of large DNA fragments can be performed simply and quickly. Therefore, YAC has become a more commonly used method in CoV reverse genetics research. Its construction process is similar to the BAC -based reverse genetics system. First, the CoV genome to be constructed is divided into several fragments with overlapping regions, and the corresponding cDNA fragments are obtained by reverse transcription PCR using the viral genome as a template. After introducing a set of cDNA fragments with homologous ends and linearized vector fragments into Saccharomyces cerevisiae, an artificial chromosome containing the full-length cDNA of the viral genome can be prepared by TAR cloning.

Compared with the several reverse genetics systems mentioned above, the reverse genetics technology based on the YAC system has the following two main advantages: First, YAC has a large gene load and can carry inserts up to 300 kb; second, Yeast-based TAR cloning is more efficient at assembling component fragments. Full-length clones can be amplified in the yeast system, providing sufficient material for further studies, saving a lot of time in preparing in vitro ligation fragments, and enabling more recombination between recombination fragments than in vitro ligation-based strategies. In the early days of the pandemic, ISENI et al. prepared a SARS-CoV-2 strain within one month based on the reverse genetics platform of YAC, and at the same time constructed a SARS-CoV-2 strain with the marker gene GFP -GFP and synSARS-CoV-2-GFP have greatly promoted the research progress of SARS-CoV-2 vaccines[10].

3 APPLICATION OF REVERSE GENETICS TECHNOLOGY IN THE FIELD OF VACCINE RESEARCH AND DEVELOPMENT

Reverse genetics can study the function of related genes and their impact on virus phenotype and traits through directional changes to the CoV genome. Through reverse genetics operations, a large number of virus resources can be quickly obtained, which greatly promotes the discovery of important virulence sites of CoV and the research on the mechanism of virus action, so as to obtain ideal attenuated strains to develop vaccines. At the same time, by adding visual markers such as GFP to the virus, real-time monitoring of virus infection can also be realized, thereby optimizing the cell and animal infection models of the virus, and providing a more intuitive and accurate way for the rapid evaluation of related vaccines.

3.1 Discovery of Attenuation Sites and Vero Cell Adaptability Sites

First, on the basis of a certain understanding of CoV replication and pathogenic mechanisms, it is possible to modify the key sites of CoV genome replication or pathogenicity without affecting On the basis of its immunogenicity, corresponding attenuated virus strains are obtained to solve the safety problem in the vaccine production process. At the same time, for the emerging CoV mutant strains, the attenuated strains of the mutant strains can be quickly obtained through the modification

of the corresponding mutation sites, which can be used in vaccine production. Secondly, on the basis of the backbone of the attenuated strain, further obtain the backbone of the human vaccine cell matrix with better adaptability, higher titer and yield, introduce the S protein of the new mutant strain or the neutralization site related to immune escape, and overcome the new Variant strains have weak adaptability, low titer and antigen production. Further, using the reverse genetics platform, it is possible to conduct more in-depth research on CoV gene function, protein structure, immune escape and pathogenic mechanism, predict the possible CoV pandemic in the future, and use reverse genetics methods to obtain corresponding information in advance. Attenuated vaccine strains to prepare for a pandemic of CoV.

3.1.1 Non-structural protein (non-structure protein, NSP)

NSP1 is the first protein encoded by CoV (α and β-CoV) infected cells, which can inhibit the expression of interferon in infected cells, thereby inhibiting the body's response to the virus immune response. Therefore, NSP1 is an important site for studying CoV infection and replication. Reverse genetics studies have demonstrated that when the 141-143 amino acid KSF of SARS-CoV-2 NSP1 is knocked out, the virus virulence will be weakened. At the same time, the innate immune response of infected cells is also restored.

NSP2 has also been reported to be associated with viral replication. When NSP2 was knocked out in MHV and SARS-CoV, viral growth and replication were attenuated. However, the mechanism of action of different CoV NSP2 on host cells is different.

NSP3 is an important component of the CoV replication/transcription complex (RTC), and it is also the largest multifunctional domain protein in CoV. Among them, papain-like protease (PLpro), ubiquitin-like domains (ubi-quitin-like domains) and ADP nucleic acid phosphatase domains (ADP-ribose-phosphatase domains) were highly expressed in different CoVs. Conservative. Reverse genetics studies have demonstrated that the pathogenicity of SARS-CoV in mice will be attenuated and the innate immune response of mice will be significantly enhanced when the cyclic ADP acid glycoprotein hydrolase activity of NSP3 is reduced. At the same time, the V787S mutation of MHV NSP3 can also lead to weakened virus virulence and enhance the immune protection of the host. Amino acid residues 590-1215 of PEDV and TGEV NSP3 have also been shown to be associated with viral immune escape. NSP4 is a transmembrane protein related to the formation of double-membrane vesicles (DMVs). When researchers remove its C-terminal domain, the replication of the virus will be affected.

NSP5, also known as 3CLpro (3C-like protease) or Mpro (mot- rypsin protease), is a crucial enzyme in the replication process of CoV, responsible for the cleavage of 11 sites on pp1a/ab, thus forming 12 mature NSPs. Reverse genetics studies have shown that the T26I and D65G mutations of MHV NSP5 confer resistance to 3CL-like protease inhibitors.

NSP6 of CoV is associated with host cell autophagy and has multiple transmembrane domains. When NSP6 in the SARS-CoV genome was knocked out, the virus was unable to form DMV necessary for replication. Researchers used reverse genetics to verify that the deletion of MHVNSP7 and NSP8 can make the virus unable to replicate, and the deletion of NSP9 and NSP10 will also affect the replication of the virus.

NSP12 and NSP13 are the core parts of CoV RTC, which act as RdRp (RNA-dependent RNA polymerase) and helicase respectively, and are indispensable for virus replication. CoV NSP14 has 5' → 3' ribonuclease (ExoN) and N7 methyltransferase (N7 MTase) activities. The former is related to the fidelity of RAN polymerase and is thought to be key for CoVs to maintain their large genomes.

CoV-encoded NSP15 has endonuclease activity and plays an important role in viral replication. It is considered to be a key site for the production of attenuated CoV strains. When the H262A mutation occurs in MHV NSP15, NSP15 will be inactivated, and its pathogenicity in the mouse model will be greatly reduced, and it can stimulate the body to produce an immune protective response. In PEDV, NSP15 is also a key virulence factor.

NSP16 has 2'-O-methyltransferase activity (2'-O-MTase), which is highly conserved among CoVs and is a noteworthy attenuation target. Reverse genetics studies have proved that D129A of MHV, D130A of SARS-CoV, D130A of MERS-CoV and PEDV (KDKEK-AAAA) can inactivate NSP16, reduce virus virulence, and stimulate the body to produce stronger type I and III type interferon response. At the same time, the NSP16 mutant can also cooperate with another mutation site in NSP1, NSP14 or S protein to produce a more stable attenuated strain.

3.1.2 Main structural protein

The S protein of CoV is a type I fusion protein located on the surface of virus particles, which is composed of two subunits, S1 and S2, and is the core molecule in the development of subunit vaccines. Some researchers constructed two chimeric viruses with the S gene exchanged with each other, and found that one strain was highly virulent and the other was avirulent, proving that the S gene is necessary for CoV virulence, but not related to CoV unique factor. Another reverse genetics study studied the role of the S gene in the pathogenesis of different generations of PEDV strains in the same way, and the results showed that, in addition to the S gene, other genes also affect the virulence of the virus. However, studies on CoVs such as SARS-CoV, TGEV, IBV, and PEDV have shown that the aggregation of virus particles on the compartment in the endoplasmic reticulum-Golgi apparatus is related to two conserved motifs (YxxΦ and KxHxx/KKxx) on the S protein, The researchers deleted or mutated these two conserved motifs in PEDV and found that the YxxΦ motif (YEVF or YEAF) triggers the endocytosis of the S protein, and the KVHVQ motif participates in the viral replication process, and the S protein and the endocytosis of the host cell The interaction between the plasmic reticulum and the Golgi apparatus; deletion of these 2 motifs significantly enhanced syncytium formation in Vero cells and reduced the virulence of the strain . The E protein of CoV is the smallest protein among the main structural proteins of CoV, which is involved in the process of CoV assembly, budding and pathogenicity. The deletion of E protein can significantly reduce the replication efficiency and virulence of the virus, but it can still form infectious virus particles. However, reverse genetics studies on MERS-CoV and TGEV showed that after E gene deletion, the full-length cDNA of the virus could not be rescued in normal cells, but it could spread between cells expressing E protein. M protein is also the main structural protein required for virus particle assembly,

and can form a complete virus-like particle (VLP) together with E protein and S protein. Both end polypeptides of the SARS-CoV M protein are immunogenic, but the immune protection effect caused by them remains to be further explored. Studies have shown that the M protein of CoV can also inhibit the production of host cell interferon. The M protein of MERS-CoV can inhibit the production of type I interferon by inhibiting the BK1-dependent phosphorylation of RF3. The N protein of CoV plays multiple functions in the viral life cycle, including regulation of viral RNA synthesis, nucleocapsid formation, and viral assembly. Expression of the N protein is essential for virus recovery from infectious cDNA. N protein can induce the body to produce a cellular immune response. When the researchers immunized mice with the recombinant virus MVA-MERS-N, the mice were able to generate stronger T cell immunity and protect the mice from MHV fatal infection. However, the fragments of amino acid residues 1-422 and amino acid residues 110-422 of the SARS-CoV N protein can produce specific antigens in mice and react with the sera of recovered SARS patients. The N protein of SARS-CoV is also an interferon antagonist, inhibiting interferon synthesis by inhibiting IRF-3 and NF-κB, interfering with TRIM25-mediated RIG-I ubiquitination or attenuating PTAC-mediated RIG-I/MDA5 Activate.

3.1.3 Accessory protein

The accessory protein of CoV plays an important role in the pathogenic process of the virus. Studies have shown that deletion of ORF3 and ORF5 from MERS-CoV and ORF8 from SARS-CoV did not affect viral replication but attenuated viral virulence. Since the accessory protein of CoV does not affect viral replication, it is also often used as a target for foreign gene insertion. It is worth noting that some accessory proteins are considered to have ion channel activity and are related to the innate immunity of host cells, which may be able to help viruses evade host cell immune surveillance and enhance virus virulence. Therefore, accessory protein-deficient CoVs are considered to be an important strategy for the production of attenuated CoV strains.

3.2 Other Reverse Genetics Techniques Can Also Be Applied to the Establishment of Virus Infection Models and Vaccine Evaluation

Some researchers used reverse genetics technology to replace ORF3 in the PEDV genome with the red fluorescent protein (RFP) gene, and obtained a recombinant virus that can effectively replicate in vivo and in vitro and mediate the onset of piglets icPEDV-ΔORF3-RFP, provides a safe and efficient platform for the study of the virus pathogenesis, tropism, neutralizing antibody and vaccine protection evaluation.

4 SUMMARY AND PROSPECT

CoV has crossed the species barrier many times, and some of them have acquired the ability of human-to-human transmission. Among them, the serious threats to human life and health mainly include SARS-CoV, MERS-CoV and SARS -CoV-2. In addition, 4 other CoVs are known to infect humans causing mild disease, including Human CoV-229E (HCoV-229E), Human CoV-NL63 (HCoV-NL63), Human CoV-OC43 (HCoV-OC43), Human CoV-HKU1 (HCoV-HKU1). Bats are generally considered to be natural hosts for a range of CoVs. Some bat CoVs also have the potential to emerge in human populations, such as the SARS-like virus SHC014-CoV in horseshoe bats. Reverse genetics technology can realize direct manipulation of the CoV genome, and it is possible to obtain ideal vaccine strains for CoV vaccine production through relevant site mutations, knockouts, or rearrangements. At the same time, using reverse genetics Genetic techniques also enable deeper studies of CoV-host interactions, host immune responses, and pathogen immune evasion strategies. Such as exploring host cell pathways affected during CoV infection, identifying CoV proteins and specific signaling pathways that can suppress host innate immunity, etc. Combining reverse genetics with genomics and structural biology can help describe current CoV populations, predict possible zoonotic CoVs, prepare for future CoV outbreaks, and facilitate the prevention and control of CoV infections strategy development.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

REFERENCES

[1]World Health Organization. Coronavirus disease(COVID-19) pan-demic.(2023-05-10) [2023-05-10]. https://www.who.int/health-topics/coronavirus#tab=tab_1.

[2] bhagat S, yadav N, shah J. Novel corona virus (CO-VID - 19) pandemic: current status and possible strategies for detection and treatment of the disease . Expert Rev Antiinfec - tive Therapy, 2022, 20 (10): 1275- 1298.

[3] Hou YJ, Okuda K, Edwards CE. SARS-CoV-2 reve-rse genetics reveals a variable infection gradient in the respiratory-tory tract. Cell, 2020, 182 (2): 429-446.

[4] Ehmann R, Kristenb C, Bankw B. Reverse genetics for type I feline coronavirus field isolate to study the molecular pathogenesis of feline infectious peritonitis. mBio, 2018, 9 (4): e01422-18. DOI: 10.1128/mBio.01422-18.

[5] Wang Mengjun, Shen Shuo. Research progress on non-structural proteins of coronavirus. Chinese Journal of Biological Products, 2022, 35 (1): 100-111.

[6] Rawson JM, Duchon A, Nikolaitchik OA. Develo-pment of a cell - based luciferase complementation assay for iden - tification of SARS - CoV - 2 3CLpro inhibitors. Viruses, 2021, 13 (2): e173. DOI:10. 3390/v13020173.

[7] Ye C, Chiem K, Park JG. Rescue of SARS - CoV - 2 from a single bacterial artificial chromosome. mBio, 2020, 11 (5): e02168-20. DOI: 10. 1128/mBio. 02168-20.

[8] Masters PS, Rottier P J. Coronavirus reverse genetics by Targeted RNA recombination. Curr Topics Microbiol and Immunol, 2005, 287 (1): 133-159.
[9] Zhang QY, Deng CL, Liu J. SARS-CoV-2 replica for high-throughput antiviral screening. J General Virol, 2021, 102 (5): e001583. DOI: 10.1099/jgv.0.001583.
[10] Ju X, Zhu Y, Wang Y. A novel cell culture system mod -eling the SARS-CoV-2 life cycle. PLoS Pathog, 2021, 17 (3): e1009439. DOI: 10. 1371/journal.ppat.1009439.