Review

Deep Learning-based Research Model of COVID-19

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Abstract
Coronavirus disease 2019 is an acute infectious pneumonia. The initial symptoms in infected patients are mostly fatigue, dry cough and fever, before gradually developing into more severe symptoms such as breathing difficulties. At present, the route of transmission, occurrence and development, vaccine research and development, and treatment methods of the new coronavirus pneumonia have not yet been fully clarified. Animal models are widely used to study and clarify the pathogenesis of various human diseases but there is currently no suitable animal model that can fully simulate the clinical manifestations of patients with new coronavirus pneumonia. Therefore, it is very important to establish and screen potentially suitable animal models for the study into the new coronavirus pneumonia. This article reviews the current research status of animal models of new coronavirus pneumonia, and compares the basis of different animal models for new corona pneumonia. The advantages and disadvantages of the research have sorted out the latest research progress of different types of animals in the new coronavirus pneumonia animal model, in order to provide references for the in-depth development of experimental animal models on novel coronavirus pneumonia.

Keywords: new coronavirus pneumonia, experimental animals, animal model

1 INTRODUCTION
Pneumonia caused by the new type of virus infection has spread rapidly around the world, which undoubtedly has brought a huge impact on global public health, medical systems, and economic development. It is extremely contagious and can be spread by droplets or contact. It has a long incubation period. At first, patients often show symptoms such as fever, fatigue, and dry cough. In severe cases, there are various complications such as dyspnea, and even worse. Most patients with
the new type of coronavirus pneumonia have symptoms are mild or common. About 14% of patients have symptoms that are severe and about 5% are critical. It is extremely contagious and can be spread by droplets or contact\textsuperscript{17,19}.

It can be seen that the pneumonia caused by the new coronavirus infection is extremely harmful, and it is important to clarify the pathogenic mechanism of the new coronavirus for the diagnosis and treatment of the disease and drug development. At present, researchers have found that the whole genome sequence analysis of the new coronavirus is highly similar to severe acute respiratory syndrome-related coronavirus. Studies have confirmed that the new coronavirus is an enveloped positive-stranded RNA virus, which belongs to the \(\beta\)-coronavirus genus and infects the human body in the same way as other viruses infect the human body: the spike protein (S protein) on its envelope needs to bind to the specific receptor angiotensin converting enzyme 2 (ACE2) on the cell surface to initiate the infection pathway\textsuperscript{11}.

In recent years, in-depth research into the pathogenesis of diseases and the mechanism of curative effects, the use of animal models is an extremely important experimental method in modern biomedical research, which helps to more efficiently understand the occurrence of human diseases, the law of development, and research and prevention measures\textsuperscript{12,13}. Now that new coronavirus pneumonia has seriously endangered human life and health, the use of animal models is of far-reaching significance for the study of new corona pneumonia\textsuperscript{14-16}.  

2 RODENT CORONAVIRUS DISEASE 2019 (COVID-19) ANIMAL MODEL  
2.1 Non-transgenic Animal Model  
2.1.1 Adenovirus Transduction of hACE2 Neocorona Sensitized Mouse  
Adenovirus vector, because of its wide host range, high proliferation efficiency, and no insertional mutagenicity, is widely used in \textit{in vitro} gene transduction. Section affiliated to Guangzhou Medical University the team of Professor Jincun Zhao of the First Hospital / State Key Laboratory of Respiratory Diseases quickly established the first non-transgenic mouse model of new corona pneumonia\textsuperscript{17-19}. The adenovirus vector expressing hACE2 under the control of the CMV promoter was used to transduce the 17CL-1 cells of mice. The transgenic method allowed mouse 17CL-1 cells to express the COVID-19 receptor hACE2 protein, breaking through the natural insensitivity of mice to the new coronavirus, and successfully constructed a COVID-19 susceptible Ad5-hACE2 mouse animal model.  

The study found that Ad5-hACE2 mice showed weight loss after inoculation with the COVID-19 strain, high replication of the virus in the lungs and severe lung consolidation\textsuperscript{20,21}. This animal model has good repeatability and overcomes the natural resistance of the mice to infection, avoiding the long modeling time and difficult operation during the production of transgenic mice, and has a higher level of virus replication compared to the transgenic mouse model. Ad5-hACE2 sensitized mouse model can be used for in-depth study of the mechanism of cell and tissue damage caused by COVID-19 and its application in therapeutics, rapid evaluation of candidate vaccines, etc\textsuperscript{22}. However, COVID-19 is non-fatal for the infection of Ad5-hACE2 mice, which is useful for the study of severe new corona pneumonia.

2.1.2 Syrian Golden Hamsters  
A large number of studies in Syrian golden hamsters have confirmed that as an experimental animal, Syrian golden hamsters can be used for the study of various diseases. It is a classic experimental animal model. Chan et al\textsuperscript{23} performed artificial infection operations on Syrian golden hamsters-nasal cavity inoculated with the \(\beta\)-CoV/ Hong Kong/VMM20001061/2020 strain (extracted from patients diagnosed with new corona pneumonia in Hong Kong). Research results show that COVID-19 can be effectively transmitted from infected hamsters through direct contact or aerosol. Vaccinated and naturally infected hamsters show symptoms similar to human mild infections: shortness of breath, weight loss, reduced activity, and lethargy. Further studies have also found that virus replication occurs in the nasal mucosa and lower respiratory tract epithelial cells; the degree of consolidation gradually deepens; COVID-19 antigen is expressed at high levels in the lungs and intestines; the number of olfactory neurons in the nasal mucosa decreases. This finding may explain the loss of smell in a small number of patients with new corona pneumonia.

In addition, there are scholars pointed out that hamsters vaccinated with new corona pneumonia may be infected\textsuperscript{24}. Close contact with hamsters occurs in a short period of time after vaccination, and the transmission rate is related to the infectious virus in the nasal fluid. Compared with mice, golden hamsters are more resistant to COVID-19. Infection is more sensitive, and it is an animal model suitable for the study of new corona pneumonia\textsuperscript{25-28}. It especially provides a new idea for COVID-19 to infect neuronal cells; and because the aerosol pathway can effectively spread COVID-19 in golden hamsters, it is important for the study of COVID-19. The transmission dynamics of COVID-19 is also of great significance; it can also be used for vaccine evaluation and other aspects.

2.2 hACE2 Transgenic Mice  
As a commonly used species in the field of basic life science research, mice have a clear genetic background and relatively thorough research\textsuperscript{29,30}. However, researchers have confirmed that as an experimental animal, Syrian golden hamsters can be used for the study of various diseases. It is a classic experimental animal model. Chan et al\textsuperscript{23} performed artificial infection operations on Syrian golden hamsters-nasal cavity inoculated with the \(\beta\)-CoV/ Hong Kong/VMM20001061/2020 strain (extracted from patients diagnosed with new corona pneumonia in Hong Kong). Research results show that COVID-19 can be effectively transmitted from infected hamsters through direct contact or aerosol. Vaccinated and naturally infected hamsters show symptoms similar to human mild infections: shortness of breath, weight loss, reduced activity, and lethargy. Further studies have also found that virus replication occurs in the nasal mucosa and lower respiratory tract epithelial cells; the degree of consolidation gradually deepens; COVID-19 antigen is expressed at high levels in the lungs and intestines; the number of olfactory neurons in the nasal mucosa decreases. This finding may explain the loss of smell in a small number of patients with new corona pneumonia.

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have found that the COVID-19 virus and SARS-CoV are the same and cannot bind to the mACE2 receptor in a natural state to infect mice. Pneumonia, because these two coronaviruses share a “door” when they infect the host, the ACE2 receptor, to initiate the viral infection route to spread the virus, hACE2 transgenic mice came into being.[31,32]

In the previously reported literature, Professor Chuan Qin’s team successfully established a hACE2 transgenic mouse animal model through microinjection technology. In this model, hACE2 protein can be expressed in the lung, heart, kidney and small intestine of transgenic mice. Compared with wild-type mice, the transgenic mice are smaller. Mice are more susceptible to SARS virus and show pathological changes closer to SARS patients. In recent years, with the rapid development of CRISPR/Cas9 gene editing technology, mice can be used to study COVID-19 only by modifying them. Researchers use CRISPR/Cas9 knock-in technology to insert the complete hACE2 cDNA into the mACE2 gene, causing the insertion of the gene to be inactivated, resulting in the normal expression of the mACE2 gene, and the inserted hACE2 gene can be expressed under the regulation of the promoter of the mACE2 gene.[33,34]

The hACE2 gene has an IRES site, and the tdTomato gene is inserted downstream to achieve co-expression; Cas9 mRNA, sgRNA and the targeting construct are injected into the mouse fertilized egg, and the hACE2 transgenic mouse COVID-19 animal model can be successfully constructed. The research on new corona pneumonia is of great significance. hACE2 transgenic mice were inoculated with the COVID-19 strain in the nasal cavity. After the infection, a continuous high viral load was detected in the lungs, trachea and brain, and interstitial pneumonia appeared. However, no deaths were observed[35].

The hACE2 mouse animal model is a good “powerful tool” for studying the pathogenesis of new corona pneumonia, pathological characteristics, vaccine development and its effectiveness evaluation. For example, Lu et al.[36] have successfully established coronavirus pneumonia. The disease and syndrome combination model of the damp disease virus attacking the lungs in mice. In this model, the performance of each index is consistent with the clinical manifestations of patients with new corona pneumonia.

It is suitable for analyzing the mechanism of action of drugs against COVID-19 and evaluating the efficacy of drugs. But the mice do not cough or sneeze, so it cannot be used to study the transmission route of COVID-19 droplets. In addition, studies have found that intragastric inoculation of hACE2 transgenic mice with the COVID-19 strain can also cause infection and further cause lung disease. This has opened a “new door” for studying the transmission route of new corona pneumonia[37].

3 NON-HUMAN PRIMATE COVID-19 ANIMAL MODEL

Rhesus monkey, also known as ordinary macaque, as a non-human primate, is an important species involved in medical and biological science research in various countries around the world. In this new corona pneumonia research work, the rhesus monkey also made a huge contribution. Nowadays, COVID-19 is still spreading further around the world, but it is still unclear whether patients diagnosed with new corona pneumonia will be infected by COVID-19 again after they are cured. In order to explore this problem, the rhesus COVID-19 animal models of reinfection came into being. Professor Chuan Qin’s team and Chandrashekhar have successfully established a rhesus monkey COVID-19 infection animal model and a rhesus monkey COVID-19 reinfection animal model[38,39].

The rhesus monkey model was infected with the new corona pneumonia for the first time during this period, there were mild symptoms similar to the human body, such as slight decreases in appetite and in activity response, but no fever, weight loss, respiratory distress or death were observed. Further studies have shown that the virus can replicate efficiently in the respiratory tract and lungs[40]. In the rhesus monkey model of new corona pneumonia reinfection, rhesus monkeys with little or no reinfection will show clinical symptoms, and the viral load will be significantly reduced compared to the initial infection. It is speculated that COVID-19 in the initial attack on a non-human primate will trigger a rapid immune response in its body, thereby protecting the host from re-infection, but the immune relevance caused by infection has not yet been known, and a large number of studies are still needed to prove the durability of natural immunity. In addition, Professor Chuan Qin’s team also inoculated the COVID-19 strain through the tracheal route, and successfully established an age-related new corona pneumonia infection rhesus monkey animal model. The interstitial pneumonia caused by the disease is more serious in elderly monkeys, confirming the clinical feature of the high mortality rate of elderly patients with new corona pneumonia, which has far-reaching significance for the in-depth study of the pathogenicity of new corona pneumonia.

In the research work of new corona pneumonia, rhesus monkeys animal models can also be used to evaluate vaccine efficacy, develop treatment plans, etc. However, the currently reported non-human primate models show only mild clinical symptoms after being infected with COVID-19, so further research and construction are needed, and the development of severe new crowns a non-human primate animal model of
clinical manifestations of pneumonia. In addition, Deng et al. [41] inoculated rhesus monkeys with the COVID-19 strain through the conjunctival route and found that the conjunctiva is also a potential route for the spread of the new coronavirus, which is to explore the spread of COVID-19. The approach provides new ideas.

4 OTHER MAMMALIAN COVID-19 ANIMAL MODELS

4.1 Ferrets

Studies on ferrets have shown that ferrets are suitable animal models for studying influenza viruses, and ferret ACE2 has been confirmed to contain key SARS-CoV binding residues, so it is appropriate to use ferrets to study new corona pneumonia. Man-infected mammalian ferrets-nasal inoculation of the NMC-nCoV02 strain (extracted from patients diagnosed with new corona pneumonia in South Korea). After confirming that ferrets are infected with new corona pneumonia, in order to evaluate the mode of transmission of the virus, separate them with the same quality. The same number of juvenile ferrets are in direct and indirect contact. Studies have found that after contracting new corona pneumonia, the mammalian ferrets show similar clinical symptoms: increased body temperature, occasional coughing, decreased activity (similar to human fatigue), virus replication, etc. Direct contact between infected ferrets and uninfected juvenile ferrets can effectively contract new corona pneumonia. COVID-19 can infect juvenile ferrets through various body fluid samples.

The newly established new corona pneumonia infection and transmission, the ferret animal model is naturally susceptible to COVID-19; it can show mild clinical symptoms similar to humans after infection. However, it still has limitations when studying new corona pneumonia. This animal model only shows mild clinical symptoms and did not appear weight loss, even death, and the virus titer in the lungs is relatively low, so it is impossible to observe, analogy, and study the clinical characteristics of moderate and severe patients with new corona pneumonia. Considering that COVID-19 can spread rapidly in the population. The high susceptibility of snow mink to COVID-19 and other characteristics, the ferret animal model will be a “powerful weapon” for studying the stage of COVID-19 infection and rapid transmission, evaluating the effects of preventive antiviral drugs and preventive vaccines.

4.2 Cats and Dogs

Domestic cats and dogs are the most commonly kept pets in most households. They are intimate with humans, eat and live together. During the period when the new corona pneumonia is spreading freely, research has confirmed that domestic pets are also infected with new corona pneumonia. Because the viral receptor ACE2 in cats and dogs is very similar to the viral receptor hACE2 in humans, it is susceptible to COVID-19, but there are no obvious clinical symptoms similar to humans.

Studies have shown that COVID-19 specific neutralizing antibodies can be detected in infected cats [42]. This is a serological study of new corona pneumonia screening of specific antibodies against COVID-19 provides new ideas. From the perspective of prevention, the isolation and virus testing of the pets of patients diagnosed with new corona pneumonia are very important for how to manage the companion pets of patients with new corona pneumonia. Although existing studies have shown that domestic cats and dogs are at risk of participating in the spread of COVID-19, a lot of research work is still needed to confirm the route of transmission of COVID-19 between humans and mammals.

4.3 Bat Intestinal-like Organisms

Bats are also known as the “natural reservoirs” of viruses. They can contain various viruses, especially RNA viruses. For humans and other animals, they have huge safety risks [43]. The existing cases of new corona pneumonia patients show that COVID-19 infections can also cause intestinal involvement in the human body. Researchers used the method of intestinal vaccination (samples from confirmed patients with new corona pneumonia in Hong Kong) to determine whether the intestines of bats are susceptible to COVID-19 [44,45].

The results show that bats intestinal tract is highly sensitive to COVID-19 infection, with cytopathic effect and efficient virus replication after infection [46,47]. Although the route of intestinal infection is unclear, it can still be considered that COVID-19 can pass through the intestinal tract propagation. Compared with Vero E6 cells, the bat gut-like organism model may isolate viruses more efficiently for subsequent research, and it may also be a “new world” for exploring the potential source of COVID-19 [48,49].

5 OUTLOOK

At present, researchers have established a variety of COVID-19 animal models, among which Chinese scientists have made outstanding contributions and laid a solid foundation for better research on new corona pneumonia. The Institute of Medical Laboratory Animals, Chinese Academy of Medical Sciences took the lead in successfully establishing transgenic mouse models and rhesus monkey models. In view of emergency needs and related requirements for the use of animal models, and at the same time, to ensure the accuracy of vaccine effectiveness evaluation, to standardize the establishment of animal models and guide the application of experimental animal models, the animal model preparation technical specifications need
to be formulated.

Nowadays, various animal models of COVID-19 have been constructed to simulate the clinical manifestations of patients with mild and moderate infections of new corona pneumonia, but they cannot simulate the clinical manifestations of patients with severe and fatal new corona pneumonia. According to literature reports, patients with severe and critical new corona pneumonia are in serious condition and have a high case fatality rate.

Therefore, there is an urgent need to prepare severe and critical COVID-19 animal models and analyze their clinical characteristics. This is useful for understanding the clinical law of new pneumonia caused by the COVID-19 virus. Although non-human primates can simulate the clinical manifestations of human neo-corona pneumonia to a high degree, they are still different from the human body itself. In order to more faithfully simulate the pathological characteristics of human neo-corona pneumonia, the next step is to establish a human source. It is very necessary to animate the immune system or animal models of humanized organs.

6 CONCLUSION

In view of the severe situation of the new crown epidemic, in order to provide a reference basis for the effectiveness of clinical emergency use of vaccines, scientific, applicable, and standardized vaccine protective animals have been formulated model preparation technical specifications, animal model preparation methods refer to standard content. According to reported literature, animal models of new corona pneumonia use a wide variety of animals and a large number, which can be roughly divided into three categories, rodents, primates and other mammals (snow mink, cat, dog, and bat). Rodents are widely used in the study of new corona pneumonia and have become a classic animal species for constructing COVID-19 animal models. Among them, researchers use CRISPR/Cas9 gene editing technology, adenovirus vector system, it breaks through the natural resistance of mice to COVID-19, making mice susceptible to COVID-19, and the results are reproducible. The advantages of rodent COVID-19 animal models are mainly manifested in clear genetic background, moderate size, and reproduction cycle is short; there are fewer ethical issues. In addition, mild clinical symptoms of COVID-19 can be shown.

However, the current rodent model for new corona pneumonia research still has limitations. This animal model does not sneeze or cough, and cannot study new corona pneumonia. The method of droplet transmission is unable to accurately replicate the pathological mechanism of COVID-19 and simulate the clinical manifestations of moderate and severe new corona pneumonia. Non-human primates have been used earlier in the study of new coronavirus pneumonia, and their evolutionary affinity with humans the highest. It is naturally susceptible to COVID-19, and the experimental results are similar to the performance of humans suffering from new corona pneumonia, but due to its high requirements for breeding conditions, more complicated experimental operations, high costs, limited numbers and many ethical issues, the new crown pneumonia research is restricted.

Other mammalian COVID-19 models are naturally susceptible to the new crown pneumonia virus and show mild symptoms after infection. They can be applied to research fields related to new crown pneumonia such as transmission routes and vaccine evaluation. These three types of COVID-19 animal models are of great significance in the process of exploring new corona pneumonia. They have their own distinct characteristics and can be applied to different research fields. Therefore, it is very important to select specific types of COVID-19 animal models according to the research content.

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Conflicts of Interest

The authors declared no conflicts of interest.

Author Contribution

Gao Y conceived and designed the study. Fan X and Chen W wrote the manuscript. Huang S participated in collecting data and helped to draft the manuscript. All authors reviewed and approved the manuscript.

Abbreviation List

ACE2, Angiotensin converting enzyme 2
COVID-19, Coronavirus disease 2019

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