

Reviews of drug candidates for COVID-19

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Abstract. The Outbreak of coronavirus disease 2019 (COVID-19) has become a global pandemic since December 2019. In order to combat the COVID-19, researchers have carried out many related experiments, including the pathogenesis of virus, to understand the structure and mechanism of the novel coronavirus. In addition, they have conducted in vitro and clinical trials for drug development. In this paper, the possible drug targets are discussed from the perspective of inhibiting virus entry into cells and inhibiting virus replication. In addition, the structure of the virus and its mechanism of entry into host cells are reviewed. Through this review, drug researchers can have a comprehensive understanding of the potential targets being investigated.

Keywords: COVID-19; SARS-CoV-2; SARS-CoVs; Spike proteins; RdRp; drug candidates.

1. Introduction

The first reported case of coronavirus disease 2019 (COVID-19) has attracted global attention since December 2019 [1]. The World Health Organization (WHO) officially announced a global pandemic status for COVID-19 [1]. The outbreak of COVID-19 has spread worldwide and precipitated into a global pandemic since December 2019, and COVID-19 has affected 219 countries [1, 2]. By isolating virus from clinical samples and sequencing their genomes, they found the genome of COVID-19 was similar to severe acute respiratory syndrome coronavirus (SARS-CoV) [3]. Therefore, the virus caused COVID-19 was named as a novel coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [3]. The coronavirus study group of the International Committee on Taxonomy of Viruses has stated that coronavirinae is divided into 4 genera: alphacoronavirus, betacoronavirus, gammacoronavirus, and deltacoronavirus [4-7]. Alphacoronavirus and betacoronavirus have the ability to cause human diseases, SARS-CoV and MERS-CoV belong to betacoronavirus [8, 9]. SARS-CoV-2 has 79.5% nucleotide sequence identity to SARS-CoV and 50% identity to MERS-CoV [10-12].

The COVID-19 is highly contagious and has the ability of infection through direct and indirect contact. The rapid transmissibility, variance and mortality of COVID-19 increased the complex and difficulty of prevention, control and treatment. As of January 12, 2021, there are more than 88 million cases of COVID-19 and 1.9 million deaths cases reported globally [1]. The epidemic has overwhelmed many countries, and there have been new confirmed cases around the world so far [1]. To treat and prevent COVID-19, there are now two main responses, developing vaccines and developing medicines. Along with the development of the scientists' research, the vaccine has been developed. In terms of vaccine development, there are different types of vaccines, including recombinant subunit vaccines, DNA vaccines, and mRNA vaccines [13]. Subunit vaccines are considered highly safe because they are expected to induce the immune system without introducing the infectious virus [13]. However, SARS-CoV-2 spike protein (S) has been proposed as the primary target in such vaccines' research so far [14]. There have been preliminary research reports on SARS-CoV-2 mRNA vaccine. Candidate vaccine mRNA-1273 (Moderna) is a lipid nanoparticles-encapsulated, nucleoside modified mRNA-based vaccine that encodes a stable SARS-CoV-2 spiking glycoprotein the prefusion conformation [15]. The serum neutralization activity was detected in 45 adults who received the vaccine during the phase I trial, demonstrating the ability of the vaccine to prevent infection of COVID-19 [16]. Nevertheless, studies have shown that long-term antibody responses may not occur, and long-term evaluation will be relevant. In addition, the safety and efficacy of vaccines need to be careful with testing and surveillance. In order to speed up the

development of vaccines, many researchers changed or shortened the steps [17]. Whether there may be unknown side effects even unknown, the study of the medicine for SARS-CoV-2 should also be considered.

Researchers test existing compounds and conduct large-scale screening to identify new compounds that effectively target SARS-CoV-2. More than 3,000 clinical trials have been registered globally, including about 500 phase 3 trials, designed to test the effectiveness of prevention and treatment of COVID-19 [18]. A large number of studies have proved that SARS-CoV-1 and SARS-CoV-2 are similar in terms of protein structure and cell entry mechanism, which provides a basis for drug repurposing. This systemic review introduces the current possible drug candidates of COVID-19 from the perspective of inhibiting the virus entry into cells and inhibiting virus replication. Also, the pathogenesis of SARS-CoV-2 is reviewed in this paper.

2. Pathogenesis Of SARS-Cov-2

2.1. Structure of SARS-CoV-2

COVID-19 is caused by an enveloped, single-stranded positive-sense RNA virus named SARS-CoV-2 [19]. There are 29,903 nucleotide bases in the RNA genome of SARS-CoV-2 with 10 open reading frames (ORF) [19]. They encode structural and non-structural proteins. The structural proteins include spike protein (S), envelope protein (E), nucleocapsid protein (N), and membrane protein (M), which is encoded by ORF2-10 [20, 21]. The non-structural proteins (nsp1-16) are encoded by ORF1ab [19]. The host infection, membrane fusion, viral assembly, morphogenesis and release of virus particles are responded by structural protein [22, 23]. The smallest protein in the viral membrane is E protein, and M protein is a transmembrane protein and is the most abundant in the viral membrane [19, 24]. M protein is a critical factor for the assembly of virus. The formation of helical ribonucleocapsid (RNP) from the viral genome RNA (gRNA) is promoted by N protein [24, 25]. S protein is responsible for receptor binding and the fusion of membrane [25, 26]. In addition, three structural proteins, E, M, and N proteins, are responsible for improving particle formation and viral assembly [22, 23, and 25].

2.2. Entry mechanism of SARS-CoV-2

Spike proteins attach to the angiotensin-converting enzyme 2 (ACE2) receptor of host and facilitate the entry of the virus into the host cell, leading to conformational changes that result in the fusion of the virus and the cell membrane [27]. Within the endocytic vesicles, pH-dependent activation of cathepsin L leads to the triggering of spines, which in turn leads to the release of the viral RNA genome into the cytoplasm [28]. Alternatively, the transmembrane protease serine-type 2 (TMPRSS2) is also involved in activating the spiking protein on the cell surface, allowing the viral genome to enter. When spike protein binds to host receptor, the conformation of heptad repeat 1 (HR1) and heptad repeat 2 (HR2) in S2 domain of S protein will change to form a six-helix bundle fusion core [28]. The change of conformation can tense the binding between host receptor and spike protein and promote the fusion of viral membrane. Once in the cytoplasm, the ORF1Ab fragment of the viral RNA genome is translated into replicase polyprotein PP1Ab and is produced by PLPRO and 3CLPRO to produce NSPs 1-16, including RNA dependent RNA polymerase (RdRp) and irrotational enzymes, which play a key role in viral replication [28]. NSPs are assembled to form replica-transcription complexes. The plus strand of the RNA genome is transcribed to produce a negative strand template for the synthesis of the new viral RNA genome [28].

3. Developing Drugs By Prevent The Entry Of Virus

3.1. Spike proteins as a potent target

There are 1273 amino acids in S protein, which consists of the N-terminal S1 domain for binding to the cellular receptor and the C-terminal S2 domain for membrane fusion [24]. The receptor-binding domain (RBD) consists of the S1 subunit that can interact with the receptor and form a trimer particle [29].

A recent study proved the designed inhibitors based on the mechanism of HR1 and HR2 could inhibit the fusion of the membrane of SARS-CoV-2 [27]. EK1 is a coronavirus fusion inhibitor by targeting the HR1 domain. Furthermore, EK1 was also found to effectively inhibit the viral membrane fusion of SARS-CoV-2 in 293 T cells [27]. According to the function of pseudovirus infection inhibition of EK-1. Xie et al. covalently link cholesterol (as EK1C) and palmitic (as EK1-P) to EK-1 with a polyethylene glycol (PEG) spacer, respectively [27]. Both EK1C and EK1P showed the inhibition of pseudovirus infection in SARS-CoV-2 [28]. In addition, the stability and effectiveness of EK1C were further proved in vivo studies [28]. Research showed that EK1C was effective in preventing the infection up to 12 h and that EK1C was able to protect the mice up to 0.5 h after infection [27]. Hence, EK1C is a potential drug candidate for treating SARS-CoV-2.

3.2. ACE2 as a potent target

ACE2 provides a gateway for the transfection of SARS-CoV-2 into host cells, which means ACE2 could be a potential target for developing drugs.

ACE2 is an ACE homologue discovered in 2000, and its gene is located on chromosome XP22 and consists of 18 exons [30]. ACE2 has only one catalytic domain, which is a zinc metallopeptidase, and its unique balance function in renin-angiotensin system has different substrate specificity. ACE2 has several roles, including catalytic activity for specific substrates. It is a negative regulator of the renin-aldosterone system, the B0 AT1 amino acid transporter, and the coronavirus (SARS-CoV) receptor [31]. ACE2 limits the adverse effects of Ang II on pro-fibrosis drugs and vasoconstrictor drugs. The hydrolysis of Ang II to Ang 1-7 can reduce oxidative stress, and Ang 1-7 has anti-fibrosis and vasodilation reverse regulatory mechanisms. Therefore, ACE2 disorder leads to increased Ang II levels and decreased cardiac function [22]. Low levels of ACE2 mRNA expression are associated with hypertension and heart failure, and reduced levels of cardiac ACE2 in hypertension and diabetic heart disease have been reported [32].

ACE2 is also recognised as a membrane protease for the binding of host receptor and the spike protein of both SARS-CoV-1 and SARS-CoV-2 [32]. Computational modeling and experiments using HeLa cells that express ACE2 protein from humans indicated that ACE2 was the receptor for SARS-CoV-2 [33]. Results also demonstrated that SARS-CoV-2 had a ten times higher affinity to ACE2 than SARS-CoV, which was consistent with the higher efficiency of infection of SARS-CoV-2 [34]. Another study indicated that the expression of ACE2 might be relative to the morbidity of COVID-19 [35]. Although the study said that the lower expression of ACE could cause the lower amount of incidence of COVID-19, the mechanism of ACE2 on COVID-19 needs to further investigate.

Ou et al. proposed that the speed of spike protein into host cells could be reduced by soluble ACE2 [36]. The soluble human ACE2 was proved as a decoy receptor that successfully suppressed the infection of SARS-CoV-2 into 293 cells [36]. Recent studies screened 312 Chinese medicinal herbs, and they found Chinese medicinal herbs could significantly inhibit the interaction between spike protein and ACE2 in vitro study [18]. Other potential molecules or peptides against spike protein include the residues of spike protein (SP-4, SP-8, and SP-10) [37]. The binding of spike protein and ACE-2 in Vero E6 cells was effectively blocked by SP-10 (residues 668-679) [37]. In addition, peptide 9626 (S residues) has the ability to inhibit the invasion of SARS-CoV-2 into 293T cells [38].

3.3. Other protease as potent targets

Infection of SARS-CoV-2 into the host cells is facilitated by the concerted action of membrane proteases. These proteases have been confirmed as potential targets, and the inhibitors of proteases have been identified as drug candidates against SARS-CoV-2.

TMPRSS2 is a protease that has the ability to affect the invasion of the virus. TMPRSS2 is expressed in the epithelial cells of the lung. TMPRSS2 promotes the fusion of viral and host cellular membranes [39, 40]. Based on the results of previous studies, an inhibitor of TMPRSS2, camostat, is able to be repurposed for the inhibition of SARS-CoV-2 entry [41].

Furin is able to promote the infection of viruses such as MERS, human immunodeficiency virus (HIV), highly pathogenic avian influenza A virus (HPAIV), Ebola, and HCoV-OC43 [42-44]. Although there is no report that furin can activate fusion proteins in SARS-CoV-1 and SARS-CoV-2 [32, 45], there is a cleavage site in the spike protein of SARS-CoV-1, and SARS-CoV-2 can be cleaved by furin [28]. Therefore, furin has the ability to prime SARS-CoV. The inhibitor of furin was proved to have the ability to activate the protein of SARS-CoV-2 and help the virus into the Calu-3 cells [46].

Some of the key processes involved in endosome proliferation and viral protein assembly require the processing of these proteins by intracellular proteases after the virus has entered the host cell. These processes are important for the replication of SARS-CoV-2. Inhibitors that target proteases can prevent virus replication. Cathepsin L is an intercellular cysteine protease. Cathepsin L inhibitors were evaluated, and the results showed the inhibitors effectively reduced the SARS-CoV-2 S pseudovirus entry by 76% [36, 47]. Therefore, it details the process of the invasion of spike protein of SARS-CoV into host cells, where there are several potent therapeutic targets, such as ACE-2, TMPRSS2, furin, and cathepsin L.

4. Blocking The Replication Of Virus RNA

4.1. RNA dependent RNA polymerase (RdRp) as a potent target

RdRp is essential for the replication of RNA from an RNA template [48]. In SARS-CoV, the tripartite polymerase complex is formed by nsp12, nsp7 and nsp8 [49]. The function of RdRp is that it drives the production of negative strand RNA, new genome molecules, and messenger RNAs (mRNAs) [49]. Results indicated that there was a 96% similarity between SARS-CoV-1 and SARS-CoV-2 [50]. Therefore, repurposing antiviral drugs from SARS-CoV-1 is an efficient method to find out the effective anti-SARS-CoV-2 drugs. Beclabuvir, as an anti-SARS-CoV-1 drug, has been proved that it has the ability of inhibition of SARS-CoV-2 [28]. Remdesivir diphosphate, as an inhibitor of anti-SARS-CoV RdRp, has been used to treat COVID-19 in clinical trials [51]. Remdesivir was an antiviral molecule for treating Ebola, and now it is used for treating COVID-19 [51]. The mechanism of viral replication inhibition of remdesivir is as a nucleoside analog targeting RdRp [52, 53]. The termination of RNA synthesis is processed by incorporation of the natural nucleotide into the RNA strand [53]. A recent study reported that remdesivir is significantly inhibit the infection of coronaviruses in epithelia cells of human lung [54]. In vitro studies, the infection of SARS-CoV-2 was inhibited in Vero E6 cells with the EC₅₀ value of 0.77 μ M [51]. In addition, the effectiveness of remdesivir on SARS-CoV-2 was proved in infected rhesus macaques [55]. Furthermore, double-blinded, randomized clinical trials of remdesivir indicated that remdesivir had more clinical benefits than the placebo group [56].

4.2. Ongoing clinical trials of RdRp inhibitors

Favipiravir is an RdRp inhibitor as an antiviral medicine for treating influenza virus [57]. Favipiravir was reported that it could be a potential treatment for Ebola [58, 59]. In addition, Favipiravir was shown that it has the ability of inhibition of replication of SARS-CoV-2 in vitro study with EC₅₀ value of 61.88 μ M [51]. An open-label non-randomized control study was conducted to

compare the effectiveness of favipiravir and lopinavir/ritonavir as a treatment strategy for COVID-19. The results indicated that favipiravir had better treatment outcomes on COVID-19 [60]. Clarification of the clinical pharmacokinetic and pharmacodynamic characteristics of favipiravir based on previous clinical trials for the treatment of influenza could be useful to determine the appropriate therapeutic dose and duration of Covid-19.

Ribavirin is a guanosine analog and is used for treating hepatitis and respiratory syncytial virus [61]. Viral RNA synthesis and viral mRNA capping are inhibited by undergoing metabolic activation to its active metabolite [61]. Previous studies showed that the inhibition of ribavirin for coronavirus, such as SARS and MERS [62-64]. The efficiency of blocking for SARS-CoV-2 was also reported in Vero E6 cells with EC50 value of 109.50 μ M [51]. Furthermore, ribavirin is now under the process of phase 3 clinical trial [51, 65, and 66].

5. Conclusion

In this systemic review, the potential targets for developing a drug against SARS-CoV-2 were discussed. Among potential targets, intercellular and extracellular proteases show the druggable target's potential ability. In addition, recent researches indicated that RdRp could be an unparalleled druggable target. Furthermore, many inhibitors of RdRp were used in clinical trials, like ribavirin, favipiravir and remdesivir.

Although a vaccine is another method to prevent or eradicate SARS-CoV-2, it will take a long time to resolve the safety issues of the vaccine and develop a vaccine with the desired protective effect before it can be deployed for mass vaccination campaigns. Therefore, as scientists develop vaccines, they are also working on antiviral drugs. Given the lengthy process of drug development, reusing approved drugs appears to be an appropriate solution to address the short-term objectives of treating SARS-CoV-2. During the evaluation of prodrugs, the efficacy of drugs against COVID-19 is the only one needed to be determined, as the safety of these drugs is known. Hence, it can save a lot of time. This article reviewed the structure and mechanism of SARS-CoV-2 and discussed some potential drug candidates. Spike protein, ACE2, and other proteases can all be targeted to prevent the invasion of the virus. If the virus enters host cells, inhibition of RdRp is an option in order to prevent replication of the viral RNA. This paper discusses the potential drug targets and the current research progress of these targets. Among them, drugs that inhibit RdRp have entered the clinical trial stage, while those that inhibit ACE2 are still in the in vitro trial stage. Based on these, drug developers can quickly narrow down the list of potential targets. Moreover, health care workers can narrow the range of antiviral drugs to screen for.

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