



Overview of Targets and Potential Drugs of SARS-CoV-2 According to the Viral Replication

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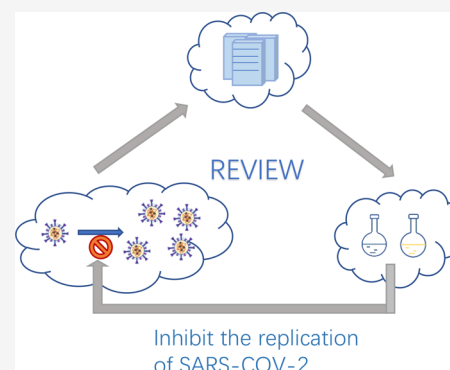
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ABSTRACT: Since the novel coronavirus pandemic, people around the world have been touched in varying degrees, and this pandemic has raised a major global health concern. As there is no effective drug or vaccine, it is urgent to find therapeutic drugs that can serve to deal with the current epidemic situation in all countries and regions. We searched drugs and response measures for SARS-CoV-2 in the PubMed database, and then updated the potential targets and therapeutic drugs from the perspective of the viral replication cycle. The drug research studies of the viral replication cycle are predominantly focused on the process of the virus entering cells, proteases, and RdRp. The inhibitors of the virus entry to cells and RdRp, such as Arbidol, remdesivir, favipiravir, EIDD-2081, and ribavirin, are in clinical trials, while most of the protease inhibitors are mainly calculated by molecular docking technology, which needs in vivo and in vitro experiments to prove the effect for SARS-CoV-2. This review summarizes the drugs targeting the viral replication process and provides a basis and directions for future drug development and reuse on the protein level of COVID-19.

KEYWORDS: SARS-CoV-2, target, drug, replication process



INTRODUCTION

In late December 2019 in Wuhan, China, several patients were diagnosed with viral pneumonia. The virus, now known as SARS-CoV-2, is characterized by its strong pathogenicity. Since the outbreak of this global pandemic disease, people around the world have been severely affected, and this pandemic around the world is extremely grim. Real-time data from the World Health Organization has shown that as of 13 November 2020, the number of global infections reached 52 177 708, and the global death toll reached 1 286 063. Therefore, finding new therapeutic targets and developing effective drugs is required for addressing this pandemic. Currently, curative drugs for SARS-CoV-2 mainly include immune-related drugs and antiviral drugs. It is well documented that SARS-CoV-2 mainly consists of the S protein, E protein, M protein, N protein, and genome. The N protein bends in a spiral shape to bind to the genome, while the S protein, E protein, and M protein bind to the viral envelope, and the S protein plays a recognition role when the virus enters the cell.¹ These proteins are very crucial for the viral replication cycle (Figure 1), and inhibiting them may block the viral replication cycle as well as treat COVID-19. Therefore, we summarize the potential targets and therapeutic drugs for the replication cycle of SARS-CoV-2, hoping to provide a reference for scientists and help to develop more drugs suitable for clinical treatment as soon as possible (Table 1).

1. ADSORPTION

The adsorption of SARS-CoV-2 to the host cells is transmitted by the S protein of SARS-CoV-2 and ACE2 or CD147 receptors on the host cell. The core domain of S protein RBD can bind with ACE2 receptors, and this process exposes the fusion site of S2, which mediates the virus entering host cells with the help of proteases such as furin and TMPRSS2.

1.1. Spike Protein

Spike protein in coronavirus plays an important role in the process of virus infecting host cells, and it mediates the attachment, fusion, and entering host cells. Spike protein consists of three parts: the large outer membrane domain, the transmembrane domain, and the intracellular tail. The receptor-binding subunits S1 subunit and S2 subunit are all located in the outland region, and they exist in two different structural conformations. S1 subunit binds to a receptor on the host cells and the virus attaches to them. Then the conformation transforms, and the membrane fusion occurs via the S2 subunit.¹ As for SARS-CoV-2, its S1 subunit

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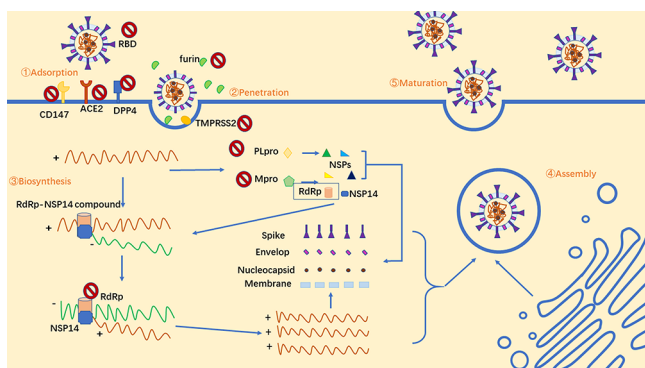


Figure 1. Replication of SARS-CoV-2. The replication process of SARS-CoV-2 is a cycle. The main processes of this cycle include adhesion, entry, biosynthesis, assembly, and maturation. (1) Adhesion. The RBD of SARS-CoV-2 spike protein binds to the ACE2 receptor, DPP4 receptor, or CD147 receptor of the target cell. (2) Fusion. The furin binding site on the target cell membrane binds to the S2 subunit of spike protein, and the virus enters the cell under the action of the TMPRSS2 enzyme. (3) Biosynthesis. The virus RNA is released into the cells, and then it is translated and cleaved into the nonstructural proteins (NSPs), such as Mpro RdRp, PLpro, which are used in the transcription and replication of the virus genome. The negative chain RNA can be used to translate all kinds of structural proteins such as E, N, S, and M protein. At this point, the biosynthesis process ends. (4) Assembly. The structural proteins, nonstructural proteins, and viral genomes assemble into the virus in the endoplasmic reticulum, Golgi body. (5) Mature. The virus is released out of the cell via vesicles and seeks out new target cells.

contains an N-terminal domain (NTD) and a C-terminal domain (CTD), which is also known as a receptor-binding domain (RBD). Recently, RBD in SARS-CoV-2 was said to be a key region to interact with receptor ACE2 in humans.² RBD is composed of a core and a receptor-binding motif (RBM), and the RBM mediates the binding between virus and ACE2. SARS-CoV-2 RBD exposes less than SARS-CoV RBD, but its binding affinity is more potent, suggesting that the structure of SARS-CoV-2 RBM strengthen it, so it can be hypothesized that RBM monoclonal antibodies can inhibit the virus from binding to ACE2.³ At present, it has been found that recombinant SARS-CoV-2 RBD fragment can bind to ACE2 and prevent the virus from entering ACE2 target cells. Besides, the SARS-CoV RBD antibody can bind to the RBD of SARS-CoV-2, and the binding force of SARS-CoV-2 RBD is stronger than that of SARS-CoV RBD. Therefore, recombinant SARS-CoV-2 RBD and SARS-CoV RBD antibodies can be used as SARS-CoV-2 virus adsorption inhibitors.⁴ It has been reported that CR-3022, a monoclonal antibody acting on SARS-CoV, can bind to the RBD non-ACE2 binding site of SARS-CoV-2, which may inhibit the binding of RBD of SARS-CoV-2 and ACE2. Therefore, CR3022 can be used alone or in combination with other drugs to inhibit the identification between SARS-CoV-2 RBD and ACE2. However, some other definite neutralizing antibodies of SARS-CoV RBD, like m396 and CR3014, fail to bind to SARS-CoV-2, though they contain domains aiming at the ACE2 binding site. This interesting phenomenon implies that the difference between the RBD of SARS-CoV-2 and the RBD of SARS-CoV is a potential target to develop novel monoclonal antibodies of SARS-CoV-2.⁵ A latest study reported that REGN-COV2, a cocktail of two potent neutralizing antibodies (REGN10987 + REGN10933) which targets nonoverlapping epitopes on SARS-CoV-2 spike

protein can prevent and treat SARS-CoV-2 infection in rhesus macaques and hamsters.⁶ Recently, a molecular docking study has found that ivermectin, an FDA-approved broad-spectrum antiparasitic drug, can bind to the RBD region of SARS-CoV-2, thereby inhibiting its coupling to human ACE2 receptor.⁷ Moreover, Nitazoxanide, an antiprotozoal drug, can inhibit SARS-CoV-2 binding to ACE2 by interfering with the high glycosylated spike proteins of SARS-CoV-2, so it inhibits the entry of the virus into its target cells.⁸ Also, one study using surface plasmon resonance found that heparin binds to SARS-CoV-2 spike proteins, which suggests that heparin may be a potential inhibitor for viral entry.⁹ Arbidol (ARB)/umifenovir is an indole-derivative molecule that functions as a virus-host cell fusion inhibitor targeting the S protein–ACE2 interaction to prevent viral entry into host cells.¹⁰ Interestingly, two independent research groups have found that Withaferin A (WFA), a steroidal lactone with anti-inflammatory and antitumorigenic properties, may bind to SARS-CoV-2 spike protein so that it may interfere with the virus entry into cells.¹¹ Also, it is reported that griffithsin can inhibit the SARS-CoV from entering host cells via binding to spike glycoprotein, but the effect of griffithsin on SARS-CoV-2 remains unknown.¹²

1.2. CD147

CD147, also known as basigin or EMMPRIN, is one of the highly glycerogelatin transmembrane proteins of the immunoglobulin superfamily, and it also acts as a main upstream stimulator of matrix metalloproteinases (MMPs). Recently, it is demonstrated to mediate the entry of SARS-CoV-2 into host cells as well as ACE2, since the spike protein of SARS-CoV-2 can bind to not only ACE2 but also CD147.¹³ As the expressions of the CD147 and MMPs in inflammatory reactions are usually high, inhibiting CD147 may have a positive effect on the severe acute respiratory syndrome triggered by SARS-CoV-2.¹⁴ Therefore, CD147 may be a potential target against SARS-CoV-2. Recently, there is a clinical trial entitled “Clinical Study of anti-CD147 Humanized Meplazumab for Injection to Treat With 2019 nCoV Pneumonia” (ClinicalTrials.gov Identifier: NCT04275245) underway in phase II in China. This trial aims to inhibit CD147 from binding to the spike protein via monoclonal antibodies and then inhibit the subsequent infection. Besides, studies have demonstrated that azithromycin could decrease the expression and action of MMP,¹⁵ but it remains unknown whether azithromycin directly changes the expression levels of CD147 receptor. Interestingly, it has been reported that doxycycline, an antibiotic analogue of tetracycline, can reduce the level of CD147 in patients with chronic periodontitis¹⁶ or a gallbladder carcinoma cell line,¹⁷ but its role in COVID-19 needs more research.

1.3. ACE2

Angiotensin-converting enzyme-related carboxypeptidase (ACE2) is a novel enzyme that was found in 2000. ACE2 mainly has 3 functions in humans: first. It can hydrolyze the carboxy-terminal leucine of angiotensin into generating angiotensin 1–9, and then the angiotensin can be hydrolyzed in smaller angiotensin peptides via cardiomyopathy in vivo or via ACE in vitro. Interestingly, ACE2 mainly expresses in the kidney and heart.¹⁸ As ACE2 has organ-specific and cell-specific expression, it plays an important part in the renin-angiotensin system of the kidney and heart. Second, it has been observed that ACE2 expresses in the lung AT2 alveolar epithelial cells, which are particularly prone to viral infection.

Table 1. Overview of Targets and Potential Drugs of SARS-CoV-2 According to Viral Replication

process	target	potential agents	molecular stocking agents	reference
adsorption	SARS-CoV-2 RBD	RBM monoclonal antibodies—CR3022; REGN-COV2; Recombinant RBD; Nidazolamide; Ivermectin Tizolozolid; Heparin; Arbidol; Withaferin A; Griffithsin		4–12
	CD147	Mepizumab; Azithromycin; Tetracycline; Doxycycline		15–17
	ACE2	Soluble recombinant human Angiotensin-converting Enzyme 2 (rhACE2); Chloroquine; Hydroxychloroquine; ACEI; Ibuprofen; DX600; MLN4760; NAAE		21–34
penetration	DPP4	sitagliptin		38
	Furin	Furin inhibitor 1; Furin convertase inhibitor (chloromethylketone); Peptidyl-chloromethylketone; α 1-PDX; Nonpeptide 2,5-dideoxycyclic vitamin; 2,5-dideoxystreptamine-derived inhibitor		46–48
biosynthesis	TMPPSS2	Camostatmesylate; Nafamostatmesylate; Bromhexine hydrochloride; Benzoselenoxanthene analogues; Nelfinavir; Cbz (Carboxybenzyl)-Phosphono-LYS(OPh) ₂	Rubitecan; Loprazolam; Compounds ZIN C000015988935; Compounds ZIN C000103558922	51–56
	Mpro	Androgapholide; Hispidin; Lepidine E; Folic; Talampicillin; Lurasidone; Ribavirin; Telbivudine; Vitamin B12; Nicotinamide; Famotidine; Pflavastatin; Oolong homobisflavan-A N3; 13b; leupeptin; hemisulfate; pepstatin A; birinapan; lyprassion; octreotide	Teicoplanin; Poziotinib; Fostamatinib; Zaprasedone; Telcagepant; Folic acid; Lurasidone; Talampicillin; Ribavirin; Telbivudine; Raltegravir; Dolutegravir; Bictegravir; Paritaprevir; Hypericin; Cyanidin-3-glucoside; Baicalin; Glabridin; α -ketoamide-11r; Cannabissin A; Isoacteoside; Darunavir	60–72
	PLpro	Ritonavir; Lopinavir; Disulfuoron	Ribavirin; Elbasvir	74–76
assembly maturation	RdRp	Remdesivir; Favipitavir; Zn(II); EIDD-2801; Sofosbuvir; IDX-184; Ribavirin	Galidesivir; Cefuroxime; Tenofovir; Penciclovir; Hydroxychloroquine; Setrobuvir; YAK; Vitamin B12	82–92
	NSP13			
	NSP14			
	E protein	Hm-methyleneamelory; PJ34		99,100
	N protein			

As mentioned above, SARS-CoV-2 RBD bind to ACE2 and then the virus entries into and fuse with the host cells. Full-length ACE2 is comprised of a C-terminal Collection-like domain (CLD) and an N-terminal PD (extracellular peptidase domain), which can recognize SARS-CoV-2 RBD via polar residues. The CLD of ACE2 ends with a 40-residue intracellular segment and a single transmembrane helix. As the CLD of ACE2 mediates homodimerization, ACE2 coupled with BOAT1 is assembled as a heterodimer.¹⁹ BOAT1, also known as SLC6A19, mediates the uptake of amino acids into intestinal cells in a sodium-dependent way. BOAT1 has to be combined with ACE2 to achieve the transfer of amino acids, which are the third function of ACE2. The above findings suggest that BOAT1 may facilitate entry of the SARS-CoV-2, and it would be attractive to speculate that the colocalized expression of these targets may play a part in the fecal–oral transmission of coronavirus.²⁰ Some drugs researching with ACE2 in COVID-19 have been somewhat controversial. A study reported that a range of ACEIs failed to inhibit the ACE2,²¹ while some research studies reported that ACEI and ARBs can increase the activity of ACE2 in the heart and kidney, block the RAS in these organs and protect them, so the application of ACEI and ARB in COVID-19 patients is good for the organs which are susceptible to RAS injury.^{17,22,23} However, the impact of ACEI and ARBs on the lung remains unknown. If they can improve the activity of ACE2 in the lung, there will be a dual function: on the one hand, the increased ACE2 can increase the susceptibility of SARS-CoV-2. On the other hand, it can ameliorate the acute lung injury induced by SARS-CoV-2.²⁴ Besides, ibuprofen, one of the nonsteroidal anti-inflammatory drugs (NSAID) has been reported that it can induce overexpression of ACE2 when used in diabetic rats.²⁵ However, a recent report demonstrates that scientific evidence does not indicate that NSAIDs will increase the infection of SARS-CoV-2. This phenomenon may be explained by the diverse functions of ACE2. However, it is worthwhile to note that the use of ibuprofen may mask the fever rise induced by COVID-19, leading to delay in the process of diagnosis and proper therapy of the infection.²⁶ There are likewise some drugs acting on ACE2 for which research is ongoing. It has been indicated that the replication of SARS-CoV-2 could be inhibited by the soluble recombinant human Angiotensin-converting Enzyme 2 (rhACE2), and rhACE2 now is undergoing a pilot study in China. Additionally, rhACE2 can reduce the serum level of angiotensin II by making the substrate of ACE2 keep away,²⁷ and the lesser level of angiotensin II can increase the activity of ACE2 so that pulmonary vascular integrity can be preserved. However, whether rhACE2 can block the SARS-CoV-2 RBD binding to ACE2 is uncertain. Chloroquine and hydroxychloroquine are 4-aminoquinoline medications that were first found to be taken up of malaria. During the COVID-19 pandemic, they were found to inhibit the SARS-CoV-2 entry into cells by increasing the pH of lysosomes as the virus entered cells via the cellular endocytosis route.²⁸ Other studies have found that chloroquine and hydroxychloroquine may block the binding of SARS-CoV-2 spike protein to ACE2 and inhibit the virus from entering target cells in the form of receptor binding by interfering with the glycosylation of ACE2²⁹ or interacting with the ganglioside at the cell surface.³⁰ In addition to the antiviral effect, chloroquine and hydroxychloroquine are applied to COVID-19 because of their anti-inflammatory and antithrombotic effects, and some scientists think that these two drugs exert

their therapeutic effects on COVID-19 through regulating iron metabolism.³¹ Therefore, chloroquine and hydroxychloroquine can be regarded as candidate drugs against SARS-CoV-2. There are still some drugs put forward to inhibit the activity of ACE2, thinking that the inhibition of ACE2 may block its binding to SARS-CoV-2 RBD. For example, DX600 is a peptide-based ACE2 inhibitor,³² while MLN4760 assumes as subnanomolar potency ACE2 inhibitors.³³ Moreover, NAAE, *N*-(2-aminoethyl)-1-aziridine-ethanamine, a small molecule inhibitor and synthetic ACE2-derived peptides, can inhibit ACE2 activity and cell fusion via the S protein of SARS-CoV-1 in vitro,³⁴ but the function of NAAE in SARS-CoV-2 still needs to be investigated. Since the function of ACE2 is multiple, ACE2-related drugs on SARS-CoV-2 often have dual effects and deserve further investigation.

1.4. DPP4

DPP4 (dipeptidyl peptidase 4), also known as CD26, is a serine protease that plays a multifunction role in human tissues.³⁵ It regulates the outcome of chemokines, cytokines, and peptide hormones and then refers to diverse diseases.³⁶ Especially since DPP4 physiologically cleaves incretin hormones, DPP4 inhibitors are always pharmacologically used for prolonging incretin half-life, potentiating meal-induced insulin secretion, and treating type 2 diabetes (T2D). Recently, structural models have predicted that the spike protein of SARS-CoV-2 can bind to the membrane-bound DPP4 via several DPP4 residues, which can target other coronaviruses entering host cells.³⁷ Besides, it is reported that blocking DPP4 receptors may potentially be beneficial in the regulation of the COVID-19 immune response, both in the early and late phases of the disease. The DPP4 inhibitor sitagliptin has been proposed as a promising therapy for patients infected with SARS-CoV-2, no matter with T2D or not.³⁸ However, the molecular study does not support that SARS-CoV-2 enters host cells via DPP4,³⁹ and a study reported that in patients with diabetes and moderate-severe COVID-19 the use of DPP4 inhibitors did not have a significant effect on mortality and clinical outcomes.⁴⁰ To sum up, there are still many important issues being solved before claiming possible beneficial effects of DPP4 on COVID-19.⁴¹

2. PENETRATION

2.1. Furin

Furin, also known as paired basic amino acid cleaving enzyme (PACE), has substrate specificity to carve up specific amino acid sequence.⁴² Furin mediates the entry of SARS-CoV-2, but not SARS-CoV. Furin can preactivate the spike protein of SARS-CoV-2 and, change the conception of the spike protein to expose the binding and fusion domains and then promote the TMPRSS2-mediated entry of the SARS-CoV-2 into the host cells.⁴³ Because of furin, SARS-CoV-2 has a higher infection rate, and some clinical patients to COVID-19 with severity may refer to it. A recent study has certified that furin carves up the spike protein efficiently and increases the affinity between spike protein and ACE2, which leads to higher pathogenicity of the SARS-CoV-2.⁴³ Besides, although it is established that furin is generally membrane-bound, a study has demonstrated that there is also a secreted form of furin, which may promisingly facilitate the cleavage of the spike protein of SARS-CoV-2 around the host cells.⁴⁴ Besides, furin has been detected in the T-cells which are activated when the body is infected by the virus. This may lead to a feedforward

loop of furin-mediated viral replication and result in a hypersensitive immunological response (cytokine storm) in some patients, which can cause lethal multiorgan failure.⁴⁵ These studies suggest that furin may be a potential antiviral target. The recent drugs inhibiting the activity of furin are furin Inhibitor I, furin convertase inhibitor (Chloromethyl ketone), and peptidyl-chloromethyl ketones.⁴⁶ Besides, a variant of naturally occurring serine protease inhibitor called α -1 antitrypsin Portland (α 1-PDX) can inhibit furin.⁴⁷ These drugs have been applied to HIV infection, so they are relatively safe. Besides, it has been reported that a nonpeptide 2,5-dideoxycyclic vitamin small molecule inhibitor could bind to furin with two sites, and this small molecule inhibitor can be considered as a potential compound against SARS-CoV-2. Moreover, scientists have designed a 2,5-dideoxy streptamine-derived inhibitor based on the crystal structure of furin, and this compound can form a complex with furin, which can inhibit the activity of it.⁴⁸ It is worth noting that furin is required for some normal developments and cellular processes, such as the regulation of blood clotting, tumor progression, and growth signaling.⁴⁹ Therefore, inhibiting furin activity for a long time may bring unpredictable risks, so brief therapy targeting furin may lead to good results in the treatment. These small molecule inhibitors and other promising orally active agents also need tests to assess their antiviral effect against SARS-CoV-2.

2.2. TMPRSS2

Coronaviruses penetrate host cells mainly via fusion. In addition to the spike protein binding to ACE2, its fusion requires cellular proteases to proteolyse the spike protein, such as cathepsins, cell surface transmembrane protease/serine 2 (TMPRSS2) proteases, and trypsin.⁵⁰ TMPRSS2 is one of the proteases mediating SARS-CoV-2 into the host cells. It can be prime not only in the cleavage of S2 but also in the cleavage of ACE2, which is required for the initiation of membrane fusion as well. The expression profile of TMPRSS2 is extensive, while the membrane fusion domain is conservative. Research has been reported that camostat mesylate, the serine protease inhibitor can impact the activity of TMPRSS2, and efficiently inhibit the virus from entering the TMPRSS2+ cells instead of the TMPRSS2- cells, while another research study reported that there is no death and other serious incidents that occur after knocking out TMPRESS2 in mice,⁴³ so TMPRESS2 can be considered as an effective therapeutic target for COVID-19. It is noteworthy that serine protease inhibitor camostat mesylate has been applied in the treatment of pancreatic inflammation disease in Japan for safety, so it can also be regarded as a promising drug for COVID-19.⁵¹ A recent research study in Japan has reported that nafamostat mesylate, a comparable drug of camostat mesylate, can inhibit the membrane fusion triggered by TMPRESS2 at a lesser concentration than camostat mesylate. This discovery indicates that nafamostat mesylate can be deemed as a potential agent for combating SARS-CoV-2. As these two drugs have been used in Japan for several years, toxicity and adverse reactions of them are known, so they can be used for COVID-19 without too much safety worry.⁵² Another potential inhibitor of TMPRSS2, bromhexine hydrochloride, which is an FDA approved agent used to expectorate and relieve cough can also be considered, although it requires clinical trials to assess its effect on the SARS-CoV-2.⁵³ Research studies on TMPRSS2 with other viruses are also worth referring. For

example, a study has found that some Benzo seleno xanthene analogues can down-regulate the expression of the TMPRSS2 gene via stabilizing the TMPRSS2 G-quadruplex structure, which is quite important for gene transcriptional activity of TMPRSS2 to inhibit influenza A virus propagation in vitro.⁵⁴ The studies above suggest that these Benzo seleno xanthene analogues may also be potential candidates against SARS-CoV-2. Also, a study has been noted that nelfinavir may inhibit cellular proteases that are required for SARS-CoV-2 fusion activation, including TMPRSS2.⁵⁵ Meanwhile, studies have shown that Cbz (Carboxybenzyl)-Phosphono-LYS(OPh)₂ irreversibly inhibits TMPRSS2 activity, so that it may be a potential drug against SARS-CoV-2.⁵⁶ Moreover, molecular docking study is designed to find drugs suppressing TMPRSS2, and a recent study identified four potential inhibitors against TMPRSS2: drugs Rubitecan and Loprazolam, and compounds ZINC000015988935 and ZINC000103558522. Nevertheless, in vitro and in vivo experiments remain to prove their effectiveness against SARS-CoV-2.

3. BIOSYNTHESIS

Replication of coronavirus need genome replication and structural protein replication, and the nonstructural protein should assist in this process. SARS-CoV-2 is a (+) SS RNA virus encoding many structural proteins and nonstructural proteins. The genome of SARS-CoV-2 includes some open reading frames (ORFs), and the first ORF is the largest one and plays a major role in the replication process of the virus. The first ORF includes ORF1a and ORF 1b and encodes nearly 70% of the entire genome. ORF1a is more conservative than ORF1b and encodes two polyproteins, pp1a and pp1ab, which can be autocatalytically processed into 16 NSPs, including two proteases (NSP3, PLpro, and NSP5, Mpro) and a viral RNA dependent polymerase (NSP12, RdRp).⁵⁷ These proteins play a significant role in viral biosynthesis.

3.1. Mpro

The main protease (Mpro, also known as chymotrypsin-like cysteine protease, 3CLpro, NSP5) is automatically cleaved from polyproteins, producing a mature protein at first. And then Mpro cleaves the downstream NSPs of pp1a and pp1ab, leading to the release of NSP4–16. Mpro plays an indispensable role in the maturation of other NSPs and promotes the biosynthesis of the virus, so we can hypothesize that the inhibition of Mpro will block the viral replication. Additionally, as there is no known homologue of Mpro in humans, the inhibition of Mpro is unlikely to cause adverse and toxic outcomes. Therefore, Mpro can be considered as a probable target against SARS-CoV-2. Recently, there are numerous studies about the detailed structure and catalytic mechanism of Mpro, which also makes Mpro be an attractive target for SARS-CoV-2. The Mpro 3D structure of SARS-CoV-2 has 3 domains, and the substrate/active site of binding is located in the cleft of domain I and domain II, consisting of HIS41 and CYS145.⁵⁸ So many agents against coronavirus are covalent Mpro inhibitors aiming at HIS41 and CYS145, but covalent inhibitors are often marked by toxicity, lower potency, adverse drug responses, and off-target side effect.⁵⁹ So high-binding-affinity noncovalent inhibitors are more suitable to treat such viral infections. However, it is worth seeing that there has been no COV-protease-specific inhibitors in clinical studies yet. Currently, several high-throughput screening methods are used to search for Mpro inhibitors, and there

are two principal types of Mpro inhibitors: one is peptide inhibitors or small-molecule inhibitors, and another is FDA approved rephrasing agents. About peptide inhibitors and small-molecule inhibitors, recent research reported that andrographolide was successfully docked in the binding site of SARS-CoV-2 Mpro, and the computational approaches also forecast that andrographolide has good solubility, pharmacodynamics property, and target accuracy.⁶⁰ Besides, a study has found that hispidin, lepidine E, and folic acid can bind tightly to the Mpro and form strong hydrogen bonds with the active site, so they can be regarded as candidate drugs against SARS-CoV-2.⁶¹ About approved drugs, a molecular docking study has found two approved drugs: Talampicillin and Lurasidone have high binding affinities to Mpro.⁶² Moreover, virtual screening of FDA approved drugs against SARS-CoV-2 Mpro found that ribavirin, telbivudine, vitamin B12, and nicotinamide can be rephrased to against SARS-CoV-2.⁶³ Famotidine, an FDA approved histamine-2 (H2) receptor antagonist applied for suppressing gastric acid secretion, was found targeting the 3CLpro and may be a potential therapeutic option for the treatment of COVID-19 in a computer-based target-drug screening of SARS-CoV-2.⁶⁴ However, another review argues that famotidine does not directly inhibit SARS-CoV-2 infection, but may activate other GPCRs.⁶⁵ Meanwhile, molecular docking studies also show an interaction between teicoplanin and SARS-CoV-2 3CLPro.⁶⁶ Further preclinical investigations are needed to estimate the inhibitory effect of teicoplanin against SARS-CoV-2. Also, a study found that some statins, especially pitavastatin, have strong binding affinity to Mpro, and even stronger than that of protease or polymerase inhibitors.⁶⁷ However, additional research is required to confirm their potent use as drugs for COVID-19. Furthermore, Oolong homobisflavan-A has been reported to be a potent inhibitor of the SARS-CoV-2 Mpro, and its effect has been proved better than the repurposing anti-HIV agents.⁶⁸ Another screening approach has found many drugs can bind with Mpro of SARS-CoV-2: 13b, one of the pyridine-containing α -keto amides defined as specific inhibitors of Mpro has been proved to display favorable pharmacokinetic properties in mice;⁶⁹ N3, as a Michael acceptor inhibitor, was shown to form a covalent bond with and to be an irreversible inhibitor of SARS-CoV-2 Mpro.⁷⁰ So these two drugs can be used as a control to find more Mpro inhibitors. In addition to the above, there are likewise some agents demonstrated to bind to the Mpro. Based on control proteins (N3 and 13b), a study has identified six potential molecules, Leupeptin Hemisulfate, Pepstatin A, Nelfinavir, Birinapant, Lypressin, and Octreotide, and these molecules can form stable interactions with hotspot residues, as these residues are conserved and can be aimed for structure- and pharmacophore-based design.⁷¹ Another two natural compounds, cannabisin A and isoacteoside, in the Vietherbs database and an HIV-1 PR inhibitor, darunavir have been found even had higher affinity to SARS-CoV-2 Mpro than 13b.⁶⁹ Besides, ebselen, an organoselenium compound with antioxidant, anti-inflammatory, and cytoprotective functions, was identified in a high throughput screening approach for finding the inhibitors of SARS-CoV-2 Mpro. It has been found in a computer-aided drug study using a silicon protein design strategy that there are many promising covalent inhibitors of SARS-CoV-2 Mpro, including poziotinib and fostamatinib (Tavalisse) (tyrosine kinase inhibitors), ziprasidone (Geodon) (an antipsychotic drug), telcagepant (MK-0974) (antimigraine drug) and folinic

acid (Leucovorin) (detoxification drug), antipsychotic drug lurasidone (Latuda) and the beta-lactam antibiotic talampicillin, the antivirals ribavirin (Copegus) and telbivudine (Sebivo, Tyzeka), the HIV integrase inhibitors raltegravir (Isentress), dolutegravir (Tivicay) and bicitegravir (GS-9883) as well as the hepatitis C virus protease inhibitor paritaprevir (ABT-450). Finally, in a molecular modeling and docking approach to determine the binding affinities to the key Cys145 and His41 residues of the active site of Mpro, the photochemical hypericin (anthraquinone derivative), cyanidin-3-glucoside (chrysin-themin), baicalin (flavone glycoside), glabridin (isoflavone), and α -ketoamide-11r were identified as potent inhibitors of Mpro.⁷²

3.2. PLpro

PLpro (Papain-like-protease), as a protease, can carve up the N-terminus of the replicase polyprotein and release Nsp1, Nsp2, and Nsp3. What is more, PLpro is essential for correcting virus replication,⁷³ so it plays an important role in the process of coronavirus replication and the infection of host cells. So it can be a promising target against the SARS-CoV-2. Ritonavir and lopinavir (Kaletra) are approved HIV-1 protease inhibitors, but the bioavailability of lopinavir is poor, and the half-life time of it is short. However, Ritonavir, as a cytochrome CYP3A4 enzyme inhibitor can slow down the metabolism of lopinavir, so combination therapy with ritonavir can significantly improve the bioavailability of lopinavir and enhance its antiviral effect in vivo. At present, the combination of the two drugs has also been used in clinical practice.⁷⁴ Moreover, a docking model has predicted that ribavirin has a strong hydrogen bond and hydrophobic interaction with PLpro, suggesting that it is likely to be a promising inhibitor of PLpro.⁷⁵ Besides, a recent study has reported that elbasvir can bind stably to not only PLpro and RdRp, helicase, so it can be predicted that it can be used alone or with other drugs like grazoprevir to block the replication of SARS-CoV-2.⁷⁶ However, the function of ribavirin on COVID-19 deserves further research.

3.3. RdRp

NSP12, referred to as RNA dependent RNA polymerase (RdRp), is a critical protease of the replication/transcription complex of coronavirus. RdRp is always conserved in coronavirus, and there exists an essential polymerase domain at the C-terminal of RdRp.⁷⁷ In the RNA synthesis, NSP8 acts as a primer, and then the NSP7-NSP8 complex increases the activity of NSP12, leading to high-binding of NSP12 to RNA.⁷⁸ Besides, studies found that targeting inhibition of the RdRp could not lead to serious toxicity and side effects,⁷⁹ so it may be a possibility for aiming at SARS-CoV-2. However, there are no specific inhibitors of RdRp demonstrated yet, and there are just a few drugs that target RdRp. Remdesivir, acting as an inhibitor of RdRp, is a nucleoside analogue. It inhibits the RNA synthesis via four ways: First, it can target the RdRp to reduce its activity.⁸⁰ Second, it can get incorporated into the replicating genome of the virus after being converted into its triphosphate form, and then attenuating replication by delayed chain termination. Third, it can evade the proofreading by viral exoribonuclease.⁸¹ Fourthly, it puts an end to the transcription of viral RNA early. Interestingly, remdesivir is a prodrug that is converted to the active drug in the triphosphate form (RTP) intracellularly.⁸² Also, remdesivir is predicted to bind with the human TMPRSS2, suggesting that remdesivir plays an important role in the coronavirus biosynthesis, and it can be

considered as an essential target against coronavirus. Nowadays, there are plenty of studies and clinical trials on it, and a network meta-analysis of 4 randomized controlled trials demonstrated that the use of remdesivir for patients with COVID-19 had a significantly higher clinical improvement rate compared with standard care alone.⁸³ Favipiravir, also known as a promising RdRp inhibitor, is a purine nucleic acid analogue which has been approved in Japan for the treatment of influenza. The chemical structure of favipiravir is pyrazine carboxamide derivative (6-fluoro-3-hydroxy-2-pyrazine carboxamide), and it is always ribosylated and phosphorylated into the active metabolite favipiravir ibofuranosyl-5'-triphosphate (T-705RTP). T-705-RTP can compete with purine nucleosides and then interferes with viral replication by incorporation into the virus RNA,⁸⁴ so favipiravir can be a potential agent against SARS-CoV-2. Recently, a meta-analysis discovered that compared with standard care, favipiravir brought significant clinical and radiological improvements, while there were few significant differences in virus clearance, oxygen support requirement, and side effects.⁸⁵ Interestingly, a new study revealed that as Zn(II) is naturally present in the structure of SARS-CoV-2 RdRp, Zn(II) may be a promising candidate to have direct inhibitory effect on the replicative cycle of SARS-CoV-2.⁸⁶ Moreover, EIDD-1931 and EIDD-2081 can inhibit the activity of RdRp, and EIDD-2081 is more promising than remdesivir, maybe because it can form two extra hydrogen bonds with the guanine base from the template strand.⁸⁷ Also, some other drugs acting on other viruses or treating other diseases are identified as having an affinity to RdRp, and may be potential drugs against SARS-CoV-2. Galidesivir can suppress SARS-CoV-2 by combining closely with the RdRp of SARS-CoV-2, and it has been pointed out that Galidesivir can establish linkages with 10 different amino acid residues. However, the effect of Galidesivir on SARS-CoV-2 has not been reported at the cellular or animal level.^{88,89} Reevaluating the anti-HCV drugs has found that sofosbuvir, IDX-184, ribavirin can be potent agents against SARS-CoV-2.⁹⁰ A computational study suggests that the methylcobalamin form of vitamin B12 may act as an effective inhibitor of the RdRp.⁹¹ Since vitamin B12 is also required for human health and has relatively small side effects, its application on COVID-19 deserved further studies. High-throughput screening in silico perspective predicted that galidesivir, cefuroxime, tenofovir, penciclovir, and hydroxychloroquine can bind to the RdRp of SARS-CoV-2 well,⁸⁹ and setrobuvir, YAK can also bind to the RdRp.⁹² The drugs mentioned above have been approved by the FDA, so they do not need toxicity measurements.

3.4. NSP13

NSP13 (Helicase) is a motor protein using the energy provided by the hydrolysis of adenosine triphosphate (ATP) to separate or rearrange double-stranded nucleic acids. The N-terminal of Nsp13 has a Zn-binding domain, and the C-terminal has a helicase domain with conserved motifs, so NPS13 can participate in the unwinding of viral double-stranded RNA along with the 5'-3' direction in a nucleoside triphosphate-dependent manner.⁹³ A recent study reported that the NSP13-dependent helical process is an essential process for SARS-CoV-2 replication, transcription, and translation, and thus NSP13 can be considered as a drug target against COVID-19.⁹⁴

3.5. NSP14

NSP14, also known as ExoN, was found both in the SARS-CoV⁹⁵ and SARS-CoV-2,⁹⁶ and it has been reported to have an exonuclease activity with proofreading function. It can correct nucleotide incorporation errors made by RdRp and then improve the fidelity of RNA synthesis.⁹⁷ Moreover, it is reported that a mutant of the MHV lacking NSP14 proofreading was extremely more sensitive to remdesivir in vitro than the wildtype virus, demonstrating that NSP14 in addition to RdRp plays an important part in virus replication. So NSP14 may also serve as a potential target against SARS-CoV-2. However, no drugs were aiming for it, so its application needs additional research.

4. ASSEMBLY AND MATURATION

4.1. E Protein

The E protein and N protein play an important role in the structural integrity and host virulence. A study has reported that the E protein, as a transmembrane protein, has an N-terminal ectodomain and a C-terminal endodomain, and it also has an ion channel activity, which is required for the pathogenesis of SARS-CoV-2.⁹⁸ And its ion channels coordinate with other viral proteins to play a role in viral assembly. So the E protein plays an important role in the assembly and release process of SARS-CoV-2. It is reported that the ion channel activity of SARS-CoV E protein can be inhibited by hm-methyleneamelory.⁹⁹ Meanwhile, PJ34, a chemical, can target the N-terminal domain of SARS-CoV E protein and thus bind to its upper RNA binding bag.¹⁰⁰ However, due to the mutation of SARS-CoV-2 relative to SARS-CoV, the effects of E proteins on SARS-CoV-2 need to be further studied.

4.2. N Protein

The N protein has two domains, the N-terminal domain, and the C-terminal domain, and each domain can bind to the RNA of the viral genome.^{101,102} The N protein has two RNA substrates: the genomic packaging signal and the TRSs (transcriptional regulatory sequence), and the C-terminal domain of the N protein can bind to the genomic packaging signal.¹⁰³ Therefore, the N protein plays an important role in the viral replication cycle. In addition, N protein is also involved in inhibiting protein translation of host cells, changing host metabolism, and apoptosis. The statement above shows that the N protein may be a possible target against SARS-CoV-2. However, there are not any approved agents to inhibit it.

■ DISCUSSION

Since the COVID-19 pandemic, people are affected to a different extent, and scientists and doctors around the world are devoted to looking for effective vaccines and drugs to control the spread of the COVID-19 and attenuate the symptoms of infection. However, there is no evidence of effective vaccines and drugs applied to the clinic. At the same time, we are facing the possibility of the COVID-19 pandemic revival, so it is urgent to search and reuse possible drugs against SARS-CoV-2, and then gradually apply them in the clinic. We searched and reviewed literatures related to SARS-CoV-2 replication cycle targets and drugs in PubMed, finding that the SARS-CoV-2 replication cycle targets are mainly in the following aspects: (1) Adhesion-related proteins. The main research focuses on the structure of SARS-CoV-2 spike

protein, and its RBD inhibitors and monoclonal antibodies, such as CR3022. Besides, ACE2, a receptor associated with adhesion, has been widely studied. As ACE2 plays an important role in the RASS (renin-angiotensin-aldosterone system), its inhibitors and ARB are also being studied, as well as the inflammatory response and prognosis of COVID-19 patients. (2) There were few studies on entry related proteins, and the studies mainly focus on TMPRSS2 protease, whose main inhibitors are camostatmesylate and nafamostatmesylate. (3) As for the virus biosynthesis, the study number of this part is large. The studies are mainly about Mpro, PLpro, RdRp, and several structural proteins. The studies about Mpro always search for potential repurposing drugs or chemicals through computer browsing and molecular docking technology. The drugs for PLpro, ritonavir and lopinavir, are undergoing many clinical trials, while the studies of RdRp are more. However, there are few studies on the assembled and mature targets and drugs in the virus replication cycle, and future studies can be carried out in these two aspects. Furthermore, in our research, it is found that the current SARS-CoV-2 targeted therapy with nanotechnology is a new research direction.¹⁰⁴ Nanoparticles can be used as drug target carriers and inhibitors to target virus-related replication proteins, so they have a good research prospect. Meanwhile, molecular docking technology will be the core technology to identify potential therapeutic drugs. In summary, this article provides a strong knowledge base about the replication cycle and drug knowledge of SARS-CoV-2 to help develop new drug candidates for the treatment of COVID-19.

CONCLUSION

In this paper, the related targets and possible drugs in each replication cycle of the virus are reviewed, which provides a basis for further development of SARS-CoV-2 drugs. For the relatively conservative proteins of SARS-CoV-2, such as entry-related protein, fusion-related protein, and RdRp, research studies mainly originate from homologous targets of other viruses. The drugs about Mpro or PLpro can be searched via large data, such as screening for repurposed drugs and related compounds. After that, in vitro and in vivo experiments should be conducted in time to evaluate the clinical effects of COVID-19. Besides, there are not enough studies on the process of SARS-CoV-2 assembly and release, suggesting that it may be a direction for finding SARS-CoV-2 drugs.

ASSOCIATED CONTENT

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