Review Article

Best insights on target molecules for drug design of nCOVID-19 based on pharmacological and peptide-based therapeutics

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ABSTRACT

Coronavirus is a non-segmented virus related to *Coronaviridae* family with an envelope and RNA that has a huge positive-sense single-stranded genome. The causative agent of COVID-19, severe acute respiratory coronavirus syndrome-2 (SARS-CoV-2) first appeared in China in 2019. The role of these viruses in pneumonia has greatly improved through molecular techniques. The virus structural proteins, viral replication factors, viral endocytosis process and enzymes are the key targets for designing drugs against COVID-19. This review considered the main molecular and cellular targets as well as peptide-based and pharmaceutical treatments for COVID-19 aiming for a better design of drugs against viruses with the best insights on key factors in this infection.

***Keywords*:** COVID-19, molecular target, cellular target, peptide

INTRODUCTION

Coronaviruses (COVs) are a group of viruses with an envelope and a huge positive-sense single-stranded genome of RNA [1-3]. The main factors of survival of viruses are depend on various agents such as surface form, suspension medium, viral

load, temperature, moisture [4-6]. The SARS CoV-2 is a causative agent of COV disease 2019 (COVID-19) which, on 11 March 2020 the World Health Organization (WHO) confirmed it as a global pandemic [7,8]. While being similar to SARS-CoV, transmissions and diagnosis of COVID-19 are very different [9,10]. The source of the virus is unidentified, but the Huainan seafood wholesale market where individuals could buy the bats was recently linked to diagnosed cases [7,11].

Negligible respiratory infections in humans are often caused by COVs, the common cold infection being one of them. However, some latest CoVs, including Middle East respiratory syndrome (MERS-CoV) and SARS-CoV, can make very serious infections [12,13].

The COVID-19 genome encodes a non-structural and great polyprotein with 30 kb size that is cut to produce 15/16 proteins, 5 attachable proteins and 4 structural proteins. The Envelope (E), the Membrane (M), the proteins of the Spike (S) surface and the Nucleocapsid (N) are necessary for gathering viruses and infection [14,15]. The S glycoprotein is a main factor because of its attachment to host cells and the host cell proteases can cleave it into a membrane-bound C-terminal S2 and an N-terminal S1 subunit [16,17]. Totally, the virus structural proteins, viral replication factors, viral endocytosis process and enzymes are the key targets for designing drug against COVID-19 [18,19].

The Food and Drug Administration (FDA) has, so far, not approved any new or successful therapies for human coronavirus SARS-CoV-2 [20,21]. This review investigated the main molecular and cellular targets as well as the pharmaceutical and peptide-based therapies of COVID-19 for a better design of drug against this virus.

Methodology

To achieve the aims of this review, the databases of Scopus, Pub Med, Google Scholar, Medline, Open Access Journals, LISTA (EBSCO) and Web of Science were exploited using the following keywords: COVID-19, molecular and cellular targets, pharmaceutical treatment, peptide-based treatment based on the most recent articles.

Molecular therapeutics

The virus proteins are molecular targets for drugs. The N protein of SARS-CoV phosphorylated by Glycogen Synthase Kinase 3 (GSK3) inhibitors and GSK3 can suppress the replication of virus in SARS-CoV-infected Vero E6 cells [22]. Molecular targeted therapy can be also

advanced to identify the S peptide for SARS and Angiotensin-Converting Enzyme 2 (ACE2) receptor. Nevertheless, the genomic mutations and the impact of antibody-dependent improvement may influence on the effectiveness of previous vaccines or prompt an ineffective immune reaction [23-25].

The Cas13a/C2c2 effector of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) related to aiming RNA has been shown to play an unplanned influence of uninhibited RNA-nuclease action together restricted target identification of RNA genes [26].

Targeting of S genes can be carried out by a accurately planned isothermic amplification primers and dynamic CRISPR guide RNAs (gRNA) [27]. The effectiveness of using oligonucleotides has been analyzed to target the RNA genome of the SARS-CoV-2, namely, Antisense Oligonucleotides (ASOs) or small interfering RNAs (siRNAs), as good treatment strategies [28].

When comparing SARS-Cov-2 genome sequences with SARS sequence, the enzymes catalytic domains like RNA-dependent RNA polymerase (RdRp) are strongly conserved in the COVs [29]. Therefore, the enzyme types and S protein could be drug favorable targets for therapeutic ideas for COVID-19 to emerge [30,31].

The development of effective broad-spectrum replication inhibitors for viruses is relevant. The other targets for viral replication of COVs are the vital proteases of virus. Such enzymes play important roles in polyprotein synthesis and the replication of viruses. The correlation of the unliganded M (pro) structures of SARS-CoV-2 with an alpha-ketoamide inhibitor is a new discovery [32].

A recent identification of a sequence of acetamides of N-(tert-butyl)-2-(N-arylamido)-2-(pyridin-3-yl) known as potential inhibitors that target SARS-CoV 3CL protease [33,34]. New inibitors of SARS-Cov 3CL protease have been designed, advanced and synthesized as mixtures containing electrophilic arylketone moiety [35].

It has been revealed that a protein as titled S-phase kinase-associated protein 2 (SKP2) is essential for poly-ubiquitination, which results in the degradation of its proteasome. Consequently, repression of SKP2 reduces replication of coronavirus while facilitating autophagy [36].

For entrance of SARS-CoV-2 to the cells, cellular proteases need to cleave the S protein at 2 places, the S protein priming, so cell and membrane of virus will be fused.

Equally via S protein, SARS-CoV-2 uses a serine protease namely Transmembrane Protease Serine 2 (TMPRSS2) for entrance to the lung cells. The camostat mesylate inhibits the SARS-CoV-2 virus entry in the cells as TMPRSS2 inhibitor [37,38].

ACE2 has been revealed to be down regulated in the presence of virus. The efficacy of recombinant ACE2 in the treatment of severe respiratory distress syndrome acute lung infections has been proven [39]. Resolvable forms of ACE2 are helpful for COVID-19 patients as shown in recent studies because of its competitive SARS-CoV S protein binders, inhibiting binding to the host cell ACE2 [40,41]. In human tongue, TMPRSS2 and ACE2 have high expression on keratinocytes. Thus, the virus can reach the alveoli by replicating in the epithelial cells of the tongue. If it does, families can get infected via distributing chopsticks or other methods. In addition, androgens regulate TMPRSS2's expression, which could be the reason why men are further vulnerable to the infection [42,43].

Altogether, the molecular mechanisms by which coronavirus invade the host cells will offer new visions into the expansion of COVID-19 therapeutic outlooks by aiming key factors like viral ACE2 factor and S protein [20,44].

DAMPs are several endogenous molecules which may be secreted or released by cytokines or stimuli of death [45]. They are many cytosolic or nuclear proteins including histones, mitochondrial transcriptions factor A (TFAM), HMGB1, family of heat shock protein, and the S100 family proteins. HMGB1 is a typical DAMP and is considered as the second plentiful nuclear protein among DAMPs. This protein is a structural factor which binds chromatin to preserve the integrity of the genome. However, inflammation and immune dysfunction are mediated by HMGB1 in response to oxidative injury, malnutrition, pathogens and hypoxia [46-48]. Thus, HMGB1 progressively becomes a desirable goal for different pathological conditions and diseases, particularly in severe diseases and septic shock [49]. Therefore, HMGB1 can performance comparable pathogenic roles in the COVID-19 infection through the mediation of immune dysfunctions and inflammation since it is considered a potential target for SARS [50].

Nitric Oxide (NO) is a crucial endogenous factor involved in many pathological and physiological procedures including relaxation of the smooth muscle, antimicrobial effects and important immune responses [51]. NO is widely

applied to treat respiratory distress and hypertension with a worthy safety outline because of its good effective on selective pulmonary vasodilation [52]. Given the high rate of pulmonary complications in patients infected with COVID-19, NO therapy may be a successful candidate for the treatment of serious COVID-19 cases by lung damage alleviation [51]. Similarly, the main factors of signaling pathways such as mammalian Target of Rapamycin (mTOR) and serine-threonine kinase (AKT) as molecular targets may also be used to treat COVID-19 infection with novel anti-proliferative drugs [53].

Cellular therapeutics

Other means for controlling COVID-19 infection are the cellular targets such as endocytosis process. Adaptor associated kinase 1 (AAK1) as a regulator factor of endocytosis may be a key candidate in targeted therapy of COVID-19 [54].

NK cells are essential immune cells that are required to defend against microbial infections, malignant or stressed cells. NK cells may wield antiviral activity in the mediation of Antibody-Dependent Cellular Cytotoxicity (ADCC) against SARS-CoV, HIV, cytomegalovirus and Herpes Simplex type 1 (HSV-1). The application of NK cell therapy to improve immunity is a plausible approach for the identification and prevention of pneumonia of COVID-19 [16]. However, in cell therapy, the induction of the regeneration of damaged cells through the use of Mesenchymal Stromal Cells (MSCs) derived from allogeneic donors is therefore an efficient method for reducing inflammation in COVID patients [55].

It is documented that stem cells hematopoietic transplantation may make possible isolation and short-term development of driven T cells related to antivirus for treating cytomegalovirus infection. Therefore, it is believed that anti-SARS-CoV-2-specific T cells could be developed as possible adjunct therapy for patients diagnosed with COVID-19 [56]. Stimulation of CD4+ T cells are quickly carried out to create Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) and other cytokines associated with inflammation specially IL-6 after COVID infection. The immunity produced by SARS-CoV-2 will decrease via blocking and inhibiting IL-6 receptors or GM-CSF [57].

The capability to create Neutrophil Extracellular Traps (NETs) is nonetheless a slight known influential role of neutrophils which contribute to organs injury and death in COVID-19. Neutrophils infiltration in lungs of COVID-19 patients has been

proved. Furthermore, prior reports have linked abnormal NET creation to secretions, pulmonary infections, thrombosis in the airways, and cytokines creation. If assumptions are right, direct and/or indirect targeting of NETs with existing drugs will minimize the clinical severity of COVID-19 [58].

Lipids are core elements of cells that play a range of physiological role spanning from a functional building block to a signaling molecule and a primary power source. The lipids play a key role in viral infection including viral replication, viral membrane fusion into the host cell, endocytosis and exocytosis. Because lipids play an key role in the life cycle of virus, thus drugs that target lipids metabolism especially statins will be valuable drugs against SARS-CoV-2 infection [4].

Peptide-based Therapeutics

Presently, reports indicate that Fc-fusion proteins of cytokine receptors can apply as an antibody-like stimulus to reduce the extremely high rates of cytokine as a treatment approach of COVID-19 infected patients [59].

The viral and cell membrane fusion is related to SARS-CoV-2 proteins including Heptad Repeat 2 (HR2) and Heptad repeat 1 (HR1). Fusion-inhibitory activity of HR2-derived peptides (HR2P) and EK1 protein (a modified OC43-HR2P peptide) have been reported to be good strategy against SARS-CoV-2 and would operate as virus fusion and cell entry inhibitors for the treatment of COVID-19 infection [60]. Lately, IPB02 – an HR2 sequence-based fusion inhibitory agent, has been produced which revealed high activity in the inhibition of COVID-19. A dual protein-based cell fusion investigation with an IC50 of 0.025μM identified the inhibitory effect of IPB02 on the cell fusion, a virus S protein-mediated cell act. A group of lipopeptides around IPB02 were formed by sequence truncation or extension. IPB02 structural activity studies have demonstrated the key roles of the N-and C-terminal amino acid motifs for their attachment and antiviral ability. Also, Circular dichroism spectroscopy has been applied as a valuable method to define the structure of cholesterol conjugated peptides. The results have indicated that the cholesterylated peptides show improved α-helical permanence [61].

Therapeutics as Immunotherapy

The Food and Drug Administration (FDA) has permitted application of blood plasma of individuals whose COVID-19 disease has been cured with a high antibody titer and can be popular donors to Convalescent Plasma (CP). CP is a standard adaptive

immunotherapy, used for prevention and treatment in several infectious diseases. This treatment by plasma might be more effective if COVID-19 patients are administered in early stages of the disease to remove the virus before it makes severe injury to the human lung [12,62].

The recovery of the COVID-19 patients can depend on developed monoclonal antibodies [63].

It has been reported that an antibody named CR3022 inhibits a highly conserved nucleotide sequence that enables SARS-CoV-2 to connect as cross-reactive. The Receptor-Binding Domain (RBD) with COVID-19 S protein CR3022 complex disclosed the characteristics of CR3022 building. The investigations have shown that CR3022 itself can target the binding epitope once the "up" orientation is the consequence of conformational differences with two RBDs on the COVID-19 S protein. This offers a mechanism and an idea for binding the antibody to the SARS-CoV-2 S protein [64].

Two monoclonal antibodies including sarilumab and Tocilizumab (TCZ) are the valued antibodies with extraordinary attraction for IL-6 cytokine receptors and have been used for treating rheumatoid arthritis [65]. Although in 2010, tocilizumab was primarily accepted by the FDA for the treatment of rheumatoid arthritis, as a corticosteroid-keeping drug for the treatment of COVID-19 and cytokine releasing syndrome after chimeric antigen receptor T-cell (CAR T cell) treatment, it has become highly common in more recent years [66].

The severity of COVID-19 disease is dependent upon enhanced inflammatory factors [TNF-α, IL-6, IL-7, IL-2, IL-1 and IL-10] [67-69], proposing that cytokine storms contribute to COVID-19 growth. IL-6 is one of the main cytokines that perturb the immune system [70]. Therefore, TCZ and sarilumab can be suspected as IL-6 receptor antagonists for application as inhibitors of systemic dysfunctional inflammation in SARS-CoV-2 patients and patients with improved condition [51].

TNF-α is also an important cytokine which mediates the host's immune response. Its inhibition results in a significant decrease in the inflammation. The two TNF-inhibitors are adalimumab and golimumab. One of the beneficial mechanisms in COVID-19 patients may be the reduction of TNF rates via the antibodies and other medications described above, which appears to be enhancing mainly in crucial phases [71,72].

Vascular Endothelial Growth Factor (VEGF) competes with VEGF receptors on

the surface of endothelial cells to bind to VEGF, and since bevacizumab is a monoclonal antibody against VEGF, this antagonism will thus inhibit the effects of binding VEGF on its receptors; for example neovascularization and cell proliferation [51]. The rates of plasma VEGF increase significantly in patients with ARDS [73]. Bevacizumab may be a potential medicinal tool for ARDS – a common problem in the severe COVID-19 disease cases [74].

The most important therapeutic drugs from the beginning until now

Various potential approaches to COVID-19 therapy are considered, including host-targeted drugs, hormone therapy, viral drugs, intestinal microecological regulators, traditional Chinese medicines, and viral vaccines [42].

Neuraminidase inhibitor – Oseltamivir - is recommended as an influenza antiviral treatment and has been broadly applied in China for COVID-19 inhibition. Also, zanamivir and peramivir are safe drugs for COVID-19 and MERS as neuraminidase inhibitors [75]. Meanwhile, Interferon-a (IFN-a) is an important therapy for preventing coronavirus replication in animals and humans [42]. Clinical guidelines propose IFN-a (5,000,000 U) as an antiviral therapy, for the current new coronavirus [42].

Furthermore, chloroquine diphosphate was identified as a possible antiviral drug with broad-spectrum [76], as it can prevent disease by enhancing endosomal pH that is essential for cell - virus fusion [77]. Owing to oseltamivir and Baloxavir antiviral action against influenza, oseltamivir has earned significant attention, and to a slighter grade baloxavir, as possible drug selections for COVID-19 [67].

While Camostat Mesylate is a confirmed protease inhibitor proven to hinder Calu-3 cells infection by SARS-CoV-2 and inhibit SARS-2 entrance by S protein into the lung cells [37], Hydroxycytidine (HC) is helpful in preventing MERS-CoV virus replication and SARS-CoV-2 disease in cultures of basic human epithelial cell of the airways [78].

As one of FDA-approved anti-parasitic drugs, 5 μM of Ivermectin added to infected Vero cells with virus has decreased viral RNA rates after 48h of culture. Thus, it could be an active drug in contrast to COVID-19 [79].

Some investigations have shown that Vitex trifolia and Sphaeranthus indicus can decrease inflammatory cytokines by the NF-kB pathway as a molecular mechanism implicated in SARS-CoV infection respiratory distress [80,81]. Similarly, protease inhibitors (lopinavir and ritonavir)

have demonstrated an effective action against MERS and COVID-19 infections [82]. The Modeling homology means have been applied to structures of two proteases of SARS-CoV-2, coronavirus endopeptidase C30 (CEP_C30), papain like viral protease (PLVP), and CEP_C30 was found to bind more devotedly to ritonavir and lopinavir, proposing that the lopinavir and ritonavir may have valuable therapeutic effects on COVID-19, probably because of their inhibitory action on CEP _C30 key factor [83]. Favipiravir is also an effective agent against COVID-19 infection (EC50 related to Vero E6 cells = 61.88μM) [84].

Lately, clinical trials of favipiravir have finalized by Chinese researchers, which display its favorable clinical effectiveness in the therapy of new coronavirus pneumonia [42]. Remdesivir (GS-5734) is another potential medication, it is an adenine-derivative prodrug and its chemical construction is almost like to tenofovir alafenamide that is the HIV reverse transcriptase inhibitor. Other postulates also revealed the interference of remdesivir with virus polymerase, while in mouse models it displays effectiveness against COVID and MERS [85]. Other studies suggested that in vitro, remdesivir has inhibitory effect on SARS-CoV-2 (EC50 for Vero E6 cells = 0.77μM) [84].

the replication of DNA and RNA viruses can be inhibited by Ribavirin– a nucleoside with strong antiviral effect, by stopping the inosine monophosphate dehydrogenase enzyme action, vital for the Guanosine Triphosphate (GTP) production [86-88]. On the other hand, Arbidol (Umifenovir), a flu-infection antiviral drug commonly used in China and Russia, when associated with arbidol mesylate have been demonstrated to have a strong inhibitory role in decreasing SARS-CoV-2 reproduction in vitro [89].

It has also been found that teicoplanin and its byproducts including telavancin and dalbavancin, block the entrance of MERS and SARS viruses. Thus, they may play an important role in suppressing the viruses that rely on cathepsin L [90,91]. Nitazoxanide which is an effective antiviral and antiparasitic medication has a broad range of in vitro antiviral activity against many viruses including rotavirus, influenza, parainfluenza, RSV, and COVs. With respect to its mechanism of action, nitazoxanide is known to have a potent antiviral effect due to its capability to interact with the host-regulated signaling pathways related to virus replication rather than the specific mechanisms of virus [92].

Quercetin and Vitamin D have been recognized as the mitigating agents of COVID-19 and can help to improve patients [93]. LJ003 and LJ001 are antiviral drugs of wide spectrum that cause both the blocking virus entrance into the host cells and degrading the viral membrane through producing oxygen single molecules [94].

EIDD-2801 is a new drug hopeful for its in-vitro effectiveness against SARS-CoV-2 virus and its outcomes from laboratories. EIDD-2801 is based on an orally ribonucleotide analog, EIDD-1931, engineered to enhance oral bioavailability and increase the uptake of drugs in non-human primates and also humans [78]. In cell investigations, EIDD-1931 sturdily inhibited replication recently of SARS-CoV-2, while a similar activity was previously reported for of MERS-CoV, SARS-CoV [51]. The EIDD-2801 proved its therapeutic and prophylactic properties in MERS and SARS murine models, displaying decreased viral lung titers, reduced weight loss, increased lung damage and enhanced lung function based on the dose and initiation time of treatment. EIDD-2801 seems more desirable drug compared to remdesivir [51].

Corticosteroids are also used for the treatment of ARDS and sepsis because of their outstanding antifibrotic, anti-inflammatory capability to inhibit deposition of collagen [95, 96]. Practical low and moderate doses of corticosteroids may exert possible therapeutic effects on patients with COVID-19 disease pneumonia [51]. Possibly other lipid-decreasing drugs and Statins – recognized inhibitors for COVID-19 infection treatment, like Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) inhibitors, are used particularly in the harshly infected individuals who are enduring diabetes and severe cardiovascular disease [97].

Artificial Intelligence (AI) technology has been used to check the drugs targeting AAK1. Among these drugs AAK1-binding compound, the Janus kinase inhibitor baricitinib, was predicted to be an appropriate potential medication of COVID-19 disease since typical doses of baricitinib drug for treatment were adequate to inhibit the main factor of AAK1 [98].

Glycyrrhizine is a dynamic component in Chinese traditional medicine (licorice root or radix glycyrrhizae, from the Glycyrrhiza glabra plant). The virus replication of SARS is inhibited by Glycyrrhizin in vitro and also this drug is applied as an alternative agent against this infection [99]. Baicalin, a huangqin-isolated flavonoid

product (Scutellaria baicalensis Georgi, Chinese skullcap), has also inhibitory effect on coronavirus in vitro [100].

Totally, the viral processing of SARS-CoV-2 inside host cells is according to the figure. This virus binds itself to the human host cell's ACE2 receptor, allowing the viral RNA to be released into the host cell, causing a cascade that eventually results in respiratory infection. This diagram often depicts selected repurposed drugs and their aim positions of effect. ACE2: angiotensin-converting enzyme 2; IL-6: interleukin 6 and TMPRSS2: type 2 trans-membrane serine protease [101].

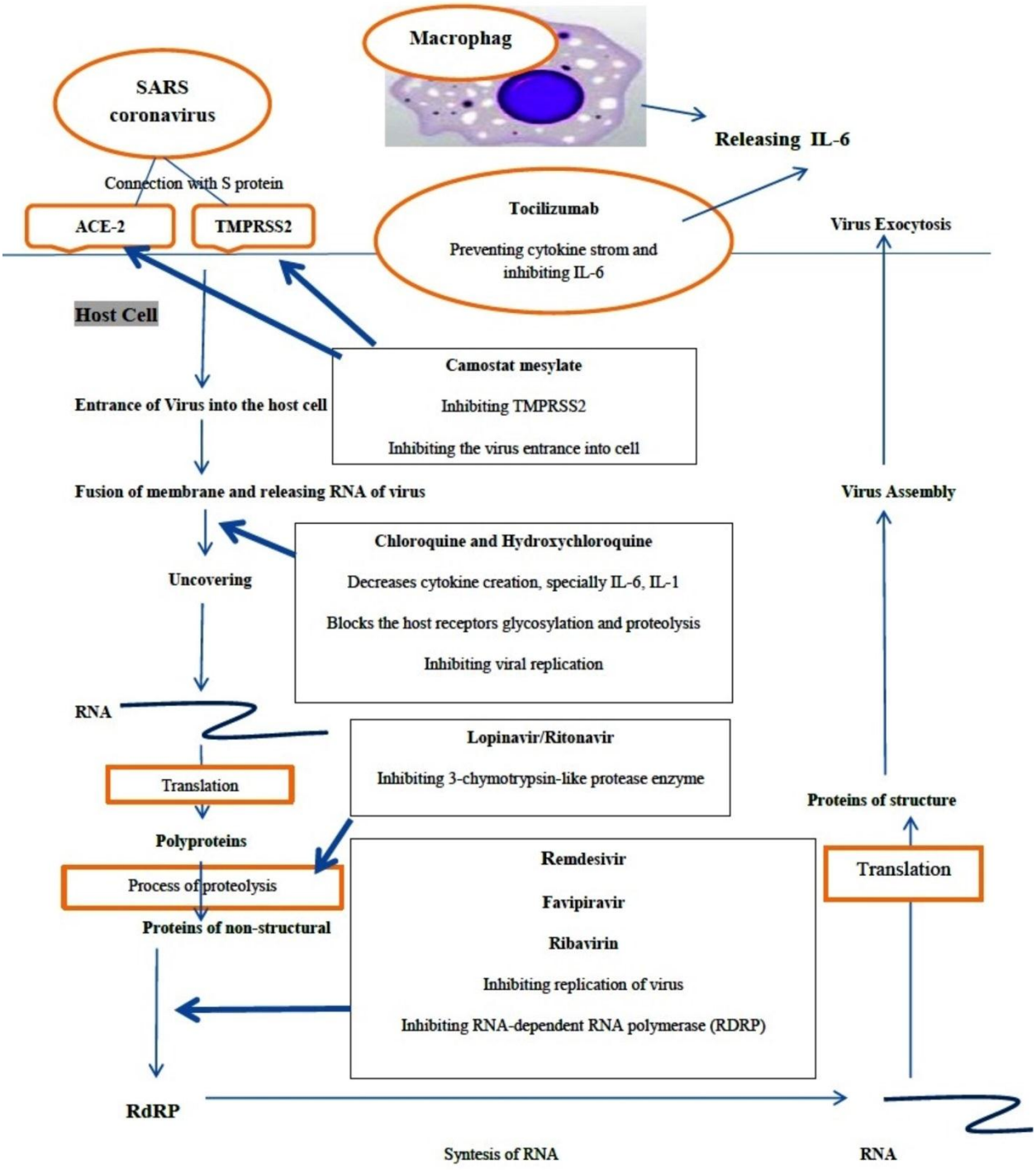

Figure 1. Schematic illustration of the entry, viral lifecycle and possible drug targets of SARS-CoV-2.

CONCLUSION

Molecular targets are key factors in the production of drugs against COVID-19. They are mainly including the virus proteins (especially S protein), replication agents and enzymes. The main factors of signaling pathways as molecular targets may also be used to treat COVID-19 infection with novel anti-proliferative drugs. Peptide and cell-based Therapeutics are also good ways in targeted therapy of COVID-19 such as proteins of HR1, HR2 on the virus and AAK1 as regulator factor of endocytosis. Therefore, targeting these factors with available drugs in the future researches can help improve patients' conditions.

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