



Potential Drug Strategies to Target Coronaviruses

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Abstract

Introduction: As the world has witnessed three severe coronavirus outbreaks in the past two decades, including the recent pandemic COVID19, caused by SARS-CoV2, it has become of utmost importance to develop drugs and vaccines against coronaviruses. The previous two outbreaks, severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) emerged in China and Saudi Arabia in 2003 and 2012, respectively. COVID19 is considered the worst of all and has taken more than 4 million lives so far and crippled the socioeconomic life of human beings in the entire world. Extensive research is being carried out to find out a solution that will not only help us to fight the current situation but also prepare us to prevent further intervention by similar viruses in the future. Here, we aim to highlight potential drug target sites in coronavirus infection or life cycle in general.

Methods: We have gone through the research papers published on coronavirus, with special emphasis on SARS-CoV, MERS-CoV, and SARS-CoV2, in peer-reviewed journals and tried to identify the possible sites in the coronavirus life cycle which can be used as potential drug targets.

Results: Studies showed that there are several unique enzymes and mechanisms involved in the coronavirus life cycle which can be manipulated to develop drugs against it. However, it has been always a challenge to develop drugs or vaccines against viruses as they utilize the host cell machinery and more difficult against RNA viruses because of their high mutation rate.

Conclusions: Effective control of the current (2020) pandemic necessarily depends on the development of either a vaccine or an effective therapeutic agent. In the past, many attempts were taken to develop vaccines after the outbreak of SARS-CoV and MERS-CoV, though no successful vaccine reached to the market as the situation came under control. In the current scenario, many laboratories have developed effective vaccines against SARS-CoV2, which have reduced both the severity of the infection and the rate of mortality considerably. However, world needs to be prepared for similar viral outbreaks in future and research must be continued to develop more effective vaccines and therapeutics against coronaviruses.

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Keywords

Coronavirus · Severe acute respiratory syndrome · SARS · Innate and adaptive immune response · Neutralizing antibody · Cytokine storm · Vaccination

Abbreviations

3CL ^{pro}	Chymotrypsin-like protease
ACE2	Angiotensin converting enzyme 2
Ang II	Angiotensin II
COVID19	Coronavirus disease of 2019
CREB1	CAMP responsive element binding protein 1
CTL	Cytotoxic T lymphocytes
DMVs	Double-membrane vesicles
DPP4	Dipeptidyl-peptidase 4
ERGIC	Endoplasmic reticulum Golgi intermediate compartment
G-CSF	Granulocyte colony-stimulating factor
HCoV-229E	Human coronavirus 229E
HKU1	Human coronavirus HKU1
HMG CoA	3-hydroxy-3-methyl-glutaryl-coenzyme A reductase
IL-10	Interleukin 10
IMP α/β	Importin α/β
IP-10	Interferon gamma-induced protein 10 (also known as CXC motif chemokine 10 or CXCL10)
MCP-1	The monocyte chemoattractant protein-1
MERS	Middle East respiratory syndrome
MIP-1A	Macrophage Inflammatory Proteins 1A
M ^{pro}	Main protease
mTOR	Mammalian target of rapamycin
M β CD	Methyl- β -cyclodextrin
NL63	Human coronavirus NL63
NNIs	Non-nucleoside inhibitors

Nsp	Non-structural proteins
OC43	Human coronavirus OC43
ORF1a	Open reading frame 1a
p53	Tumor suppressor p53
PL ^{pro}	Papain-like protease
RaTG13	Bat coronavirus <i>RaTG13</i>
RBD	Receptor binding domain
RdRp	RNA dependent RNA polymerase
RTC	Replicase-transcriptase complex
SARS	Severe acute respiratory syndrome
SMAD4	Mothers against decapentaplegic homolog 4
STST1	Signal transducer and activator of <i>transcription</i> 1
TLR	Toll like receptor
TMPRSS11D	Transmembrane protease, serine 11D
TMPRSS2	transmembrane protease serine 2

7.1 Introduction to Coronavirus

The family *Coronaviridae* is composed of several groups such as α -CoVs, β -CoVs, γ -CoVs, and δ -CoVs, which vary in their genetic content and variation in their antigenic properties. Human α and β coronaviruses such as 229E, NL63, OC43, and HKU1 are common and cause COVID19 like symptoms (Li and Luk 2019). The first human coronavirus strain, 229E, was isolated by Dorothy Hamre in the 1960s. SARS-CoV2 bears about 79% and 50% sequence similarity with SARS-CoV, a lineage B beta-coronavirus, and MERS-CoV, a lineage C beta-coronavirus, respectively (Lu et al. 2020; Mousavizadeh et al. 2020). SARS-CoV2 has almost 96% similarity with Bat-CoV RaTG13 and 88% identity to Bat-SL-CoVZC45 and Bat-SL-CoVZXC21 (SARS-like coronaviruses in bat), collected in 2018 in Zhoushan, eastern China (Lu et al. 2020). These viruses can cross the species barriers and mainly infect mammalian and avian species.

The members of *Coronaviridae* are positive-sense, single-stranded RNA viruses with envelope. The genome length varies between 27,000–32,000 bases among different coronaviruses. The envelope derived from host cell membranes carries three viral structural proteins, namely, membrane protein (M), spike protein (S), and envelope protein (E). The RNA genome is wrapped by N proteins to form the nucleocapsid inside the envelope (Siu et al. 2008). Trimeric S proteins form a crown-like spike on the viral envelope and responsible for initiating the infection (Fang Li, 2016). M protein is abundant in the viral envelope, while E protein is the smallest and is found in a small proportion in the envelope (Walls et al. 2020; Schoeman et al., 2019).

7.2 Infection Cycle of Coronavirus

Coronaviruses enter the human system through upper respiratory tract, i.e., nose, mouth, and mucous membrane of eyes and travel down through the throat and bronchial tubes toward the lungs. The initial symptoms are mild and like common flu or cold, which include cough, sore throat and runny nose, difficulty in breathing, headache, and fever. Sometimes fewer common symptoms like diarrhea, conjunctivitis, loss of smell or taste, a rash on skin, or discoloration of fingers or toes may appear. Later, extensive inflammation in the mucous membranes of airways leads to the damage of the air sacs. The sacs are filled up with fluids, pus, and dead cells leading to pneumonia (Lee et al. 2020), and patients find difficulty in breathing and require ventilator support. In case of SARS-CoV2 infection, patients with diabetes and heart diseases are at a high risk of mortality as total lung function collapses. SARS-CoV2 can infect the gastrointestinal tract and enter the bloodstream too and infect other organs like the heart, kidneys, liver, etc. (Wang et al. 2020; Naicker et al. 2020). As the endothelial cells under the epithelial cells of airway get damaged, small blood clots appear leading to stroke as a second attack. Hence, SARS-CoV2 is associated with venous and arte-

rial thrombosis and acute cerebrovascular diseases too (Chen et al. 2020).

Trimeric S proteins present on the viral envelope are responsible for initiating infection by binding to specific receptor (Gui et al, 2017). SARS-CoV and SARS-CoV2 utilize the transmembrane protein, angiotensin converting enzyme 2 (ACE2) to enter the cell, while MERS-CoV binds to an 110KDa glycoprotein, an exopeptidase called Dipeptidyl-peptidase 4 (DPP4). The length of the S proteins varies between 1160–1400 amino acids among coronaviruses. The protein is cleaved by host proteases at a multibasic cleavage site between S1 and S2 domains (S1/S2) (Du et al. 2009). S1 subunit has the receptor binding domain (RBD) which recognizes specific receptors, and the hydrophobic fusion loop or fusion peptide in S2 subunit helps in the host-viral membrane fusion (Wong et al. 2004). The nature of the protease that cleaves the S glycoprotein varies with the strain of the coronavirus. The S proteins of SARS-CoV and SARS-CoV2 are cleaved by transmembrane protease serine 2 and 11D (TMPRSS2 and TMPRSS11D) just after recognizing the receptor. However, the S protein of MERS-CoV is processed intracellularly by furin proteases before exit from the infected cell (Shulla et al. 2010; Hoffmann et al. 2020). Thus, newly released MERS-CoV particles are ready to enter new cells. In contrast, S proteins of SARS-CoV remain in an uncleaved condition upon virus release from cells. Interestingly, SARS-CoV2 has been found to contain the furin-like cleavage site at S1/S2, which is not present in the same clade of coronavirus though present in human coronaviruses OC43, HKU1 (Coutard et al. 2020). However, whether the S protein of SARS-CoV2 is cleaved or not inside the host cell and how does it depend on the TMPRSS2 mediated cleavage before entry into a cell need more studies (Hoffmann et al. 2020).

Inside the endosome, the S2 domain is further cleaved into S2' by another protease, cathepsin, and activates membranes fusion within endosomes (Miao Gui, 2017). Following endosomal membrane fusion, the viral RNA enters the cytoplasm and is translated. Two open reading frames

1a and 1ab (ORF1a and ORF1ab) are translated to produce polyproteins pp1a and pp1ab. Two proteases, M^{pro} (Main protease) or 3CL^{pro} (picornavirus 3C protease like cleavage-site specificity) and PL^{pro} (papain like protease) encoded by ORF1a cleave the polyproteins in at least 11 sites to produce non-structural proteins (nsp) (Gorbalenya et al. 1989). These proteins join to form the RNA replicase–transcriptase complex (RTC) which produces the complementary –ve sense RNAs of the +ve sense genome during replication. Discontinuous transcription gives rise to multiple sub-genomic RNAs encoding the structural proteins.

The N proteins encapsidate the genomic RNA and make the nucleocapsids in the cytoplasm. The nucleocapsids then travel through the lumen of the endoplasmic reticulum Golgi intermediate compartment (ERGIC) or vesicular-tubular cluster and acquire their membrane envelope (Ujike et al. 2015). The M proteins interact among themselves and take part in the formation of the envelope. The M proteins also help in retention of S proteins in the ERGIC and mediate its incorporation into new virions. The E proteins are abundantly produced and localized to the ERGIC where they participate in the assembly of complete virus particles.

The newly formed infectious virions are then released through the secretory pathway of the infected host cell and are ready to attack new host cells. The infection induces a cellular stress condition known as the unfolded protein response mediated primarily by viral S protein.

7.3 Role of Our Immune System

The duration of infection by SARS-CoV2 can be divided into broadly three phases. Stage I or early infection phase is called the viral response phase. In this stage, upon entry of the virus particle, the phagocytic cells of our innate immune system such as macrophages, neutrophils, dendritic cells, natural killer cells try to engulf and remove it. These cells release several cytokines, chemokines and initiate the process of inflammation. At the same time, peptide fragments generated from the

virus particle are presented by the antigen presenting cells (macrophages, dendritic cells, and B-lymphocytes) to the T-lymphocytes, both T helper and T-cytotoxic. Within 7–10 days after infection, activation of B- lymphocytes and cytotoxic T lymphocytes (CTL) lead to the production of antibodies and memory cells. Neutralizing antibodies prevent the attachment of newly produced virus particles to host cells and effector Tc cells kill the viral antigen presenting cells. Now, the immune response works with both innate and adaptive branches and slowly leads to the recovery of the patient. This is the second phase, viral load decreases and most of the people recover on their own without showing much symptoms. Activation of adaptive response clears the virus more quickly and efficiently than innate response and thus the recovery time of an infected person depends on his immune system. It has been found in case of older people and people with co-morbidities, adaptive immune response is weaker, and the virus persists for long. It generates huge amount of cytokines and chemokines which ultimately lead to ‘Cytokine storm’ and multi-organ failure. This is the third phase of the infection also called the hyperinflammation phase. Patients need the help of ventilators and some succumb to death.

7.4 Difficulty in Designing Drugs Against Viruses

Designing of drugs against viruses is very difficult as they use host cell surface proteins having specific physiological roles as the receptors to enter the cells. Upon entry into the cell, the viruses capture the host cell machinery to make progeny virions. Hence, targeting any intermediate step(s) in the viral life cycle would interfere with the host machinery leading to severe side-effects. The only way to prevent viral infection without interfering with the host system is through vaccination in which neutralizing antibodies developed against the viral surface proteins inhibit the attachment of the virus to its receptor. Moreover, high mutation rate of RNA-dependent RNA polymerase makes it more difficult to design drugs against RNA viruses.

7.5 Different Approaches to Prevent and Cure Coronavirus Infection

1. *Prevention of initiation of an infection by blocking entry of coronavirus into the target cells.*

Development of Vaccines Against Coronaviruses Literature survey shows that serious attempts were made to develop vaccines against SARS-CoV and MERS-CoV after the outbreaks in 2003 and 2012, respectively. Several groups have reported their findings on development of vaccines against SARS-CoV between the year 2003 and 2007. The strategies include use of attenuated vesicular stomatitis virus expressing spike protein of SARS-CoV, live attenuated SARS-CoV with deletion of E and other proteins, inactivated SARS-CoV, recombinant S2 fragment, B cell epitopes of S2 spike protein, trimeric recombinant spike protein, recombinant adenovirus with N terminal segment of S1 gene, DNA vaccine, etc. (Bukreyev et al. 2004; Kapadia et al. 2005; Netland et al. 2010; Tsunetsugu-Yokota Y 2008; Zhao et al. 2005). Similarly, there are reports on development of vaccines against MERS-CoV between the year 2012 and 2020, which include use of inactivated MERS-CoV, S1 subunit vaccines, recombinant adenovirus encoding the S1 subunit, DNA vaccine, virus-like particles, etc. (Folegatti et al. 2020; Muthumani et al. 2015; Kato et al. 2019).

Vaccine development strategy against SARS-CoV2, includes inactivated virus vaccine, recombinant viral vectored vaccine, protein subunit vaccine, mRNA vaccine, adenoviral vector vaccine, and recombinant influenza viral vector vaccine are prominent (Sharpe et al. 2020).

Vaccination generates neutralizing antibodies against viral surface proteins along with other protective mechanisms like activation of T cells. The antibodies attach to the viral surface and facilitate its phagocytosis and consequent removal from the host system.

Blocking of ACE2 Receptors to Prevent Viral Entry As SARS-CoV attach to the membrane bound protein ACE2 present on many cell types, blocking of this receptor can be a way to prevent the viral entry. ACE2 plays an important role in Renin angiotensin system (RAS) and its main function is to cleave Angiotensin II (Ang II) to angiotensin 1–7 which has opposite actions of Ang II. The level of Ang II has been found to be doubled in ACE2 deficient mice, while the levels of Ang 1–7 are almost undetectable. ACE2 has beneficial and protective role against tissue injury, cardiovascular diseases, hypertension, etc. Thus, blocking of ACE2 might have severe side effects and can increase susceptibility to inflammation, cell death, and eventual organ failure. Some small molecules have been identified by researchers which can act as inhibitors of ACE2, like lividomycin, which is an aminoglycoside antibiotic, burixafor, a chemokine receptor type 4 antagonist, quisinostat, a histone deacetylase inhibitor, fluprofylline, spirofylline, and diniprofylline as phosphodiesterase inhibitors, pemetrexed, an antifolate, edotecarin, a topoisomerase I inhibitor, N-(2-aminoethyl)-1 aziridine-ethanamine, etc. (Terali K et al. 2020; Markus H et al. 2020; Huentelman et al. 2004). These molecules are engaged with ACE2 through ionic interactions and can block the interaction with SARS-CoV.

7.6 Inhibition of Host Cell Proteases

Inhibition of TMPRSS2 The protease, TMPRSS2, that cleave the S protein of CoVs, is a 492 amino acid Type II transmembrane serine protease. It is abundant throughout the human respiratory tract cells and can be a potential drug target (Hoffmann et al. 2020). Though the cleavage of S protein of MERS-CoV takes place inside the host cell, it still depends on TMPRSS2 mediated cleavage and addition of trypsin with MERS-CoV increases its infectivity (Shirato et al. 2013). Therefore, inhibitors of serine proteases could be

employed for blocking the entry of coronavirus, though the major question that remains is the bio-availability of the drug in the lungs. One such serine protease inhibitor is Camostat mesilate, which blocks TMPRSS2 activity and has been approved in Japan for human use for a long time against chronic pancreatitis, reflux esophagitis, etc. (Uno Y 2020) (Fig. 7.1).

Inhibition of Cathepsin Fusion between viral envelope and endosomal membrane is dependent on endosomal protease, Cathepsin-mediated cleavage of S2 into S'. Hence, use of inhibitors of Cathepsin can block the membrane fusion and release of viral RNA into cytosol. Several protease inhibitors have been analyzed and identified which can inhibit this cysteine protease inside the endosomes (Simmons et al. 2005) (Fig. 7.1).

Inhibition of Furin and Furin-Like Proteases The protease furin is a cellular endopeptidase which is involved in proteolytic activation of many proproteins in the secretory pathway. Furin cleaves at multibasic consensus sequences having arginine residues. Many bacterial and viral proteins having furin-like cleavage sites are processed by this protease inside the cell which leads to virus propagation. Among these viral proteins, hemagglutinins of highly pathogenic avian influenza virus, surface glycoproteins of the HIV, Ebola, Marburg, and measles virus are important. Thus, blocking the furin enzyme can be a potential target in the control of many viral life cycles. The first furin inhibitor was chloromethyl ketones and many more inhibitors were developed later like α_1 -antitrypsin Portland, mutated forms of Eglin c, 83-mer prodomain of furin produced synthetically, various types of oligopeptides, and small molecule inhibitors, etc. Nona-D-arginine, a polyarginine, inhibits furin with high efficiency (Becker et al. 2012) (Fig. 7.2).

7.7 Prevention of Endosomal Membrane Fusion and Release of Viral Genome into the Cytosol

Chloroquine or hydroxychloroquine, an anti-malarial agent is known to increase the endosomal pH and prevent virus-host cell membrane fusion leading to blocking of release of viral RNA into the cytosol (Wang et al. 2020). As endosomes are involved in protein trafficking and glycosylation, increase in pH interferes with the glycosylation process of ACE2 inside them. Improperly glycosylated ACE2 does not act as receptors and prevent the interaction between virus and host cell (Vincent et al. 2005) (Fig. 7.1).

7.8 Inhibition of Viral RNA Polymerase

The activity of the RNA-dependent RNA polymerase (RdRp, nsp12) enzyme can be blocked by using nucleoside analogs which are structurally similar to dNTPs or rNTPs except the 3' carbon -OH group. Incorporation of dNTPs terminates chain elongation during replication or transcription. One such antiviral drug is Remdesivir, a nucleotide analog whose active form is incorporated into the growing RNA chain by RdRp, escapes the proofreading activity by viral exoribonuclease (ExoN) and prevents viral propagation by terminating chain elongation. Remdesivir and its other derivatives have been found to be useful against many RNA viruses like influenza, Nile virus, yellow fever virus, etc., though several various side effects like occasional myopathy, neuropathy, pancreatitis, nephrotoxicity, etc. (Khungar et al. 2010) are associated with their use (Fig. 7.2).

RdRp can be inhibited by non-nucleoside inhibitors (NNIs), which bind to conserved sequences of RdRp and change its conformation required for polymerase activity. These mole-

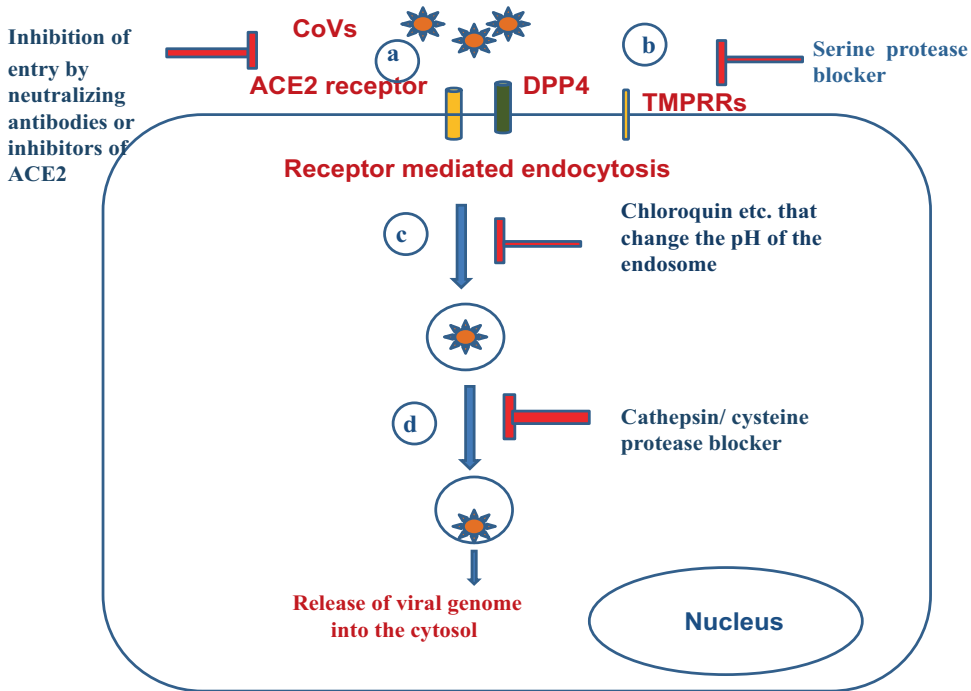


Fig. 7.1 Early stages in viral life cycle can be targeted for drug development

Different sites in coronavirus life cycle have been shown where drugs can be administered. (a) The entry of the virus can be prevented by generating antibodies through vaccination or by using inhibitors of ACE2; (b) entry can be further

blocked by targeting the cell surface co-receptor DPP4 and protease TMPRSS; (c) after receptor mediated endocytosis of the virus particle, changing of endosomal pH can prevent the release of the viral RNA into the cytosol; (d) action of another protease cathepsin required for membrane fusion can be blocked by suitable agents inside the endosome

cles are not competitive inhibitors of nucleotides and have least side effects on the host. The disadvantage is that a single mutation in the NNI binding site on RdRp can lead to drug resistance. The NNIs used for hepatitis C virus treatment include benzimidazole, indole derivatives, benzothiadiazine, thiophene-2-carboxylic acids, dihydropyranones, etc. (Chan et al. 2004). An anti-influenza virus drug, Avigan, which selectively inhibits the RNA polymerase is under phase III clinical trial against SARS-CoV2.

Replication of MERS-CoV has been found to be inhibited by Saracatinib, a 5, 7-substituted anilinoquinazoline, inhibitor of protein tyrosine kinases. It interferes with early events in the viral life cycle as found in in vitro studies (Shin et al., 2018a, b). Saracatinib also exhibits synergistic effect with anticancer drug, Gemcitabine, having antiviral activity against RNA viruses.

7.9 Inhibition of Viral Protease

The main protease, M^{pro} or $nsp5$ can be a potential target for drug development against coronaviruses with minimal toxic effects on host as similar cleavage specificity is not found in human proteases. M^{pro} shares spatial/structural similarity with the active site of HIV protease and is inhibited by anti-HIV drugs Lopinavir/Ritonavir. Many groups have proposed ligands based on in silico studies, which can interact with the protease and stop its action (Zhang et al. 2020). Some of the compounds include antibiotic Colistin, antitumor drugs like Valrubicin, Epirubicin, Vapreotide, Aprepitant, antirhinitis drug Bepostatine, etc. (Liu and Wang 2020). Nitazoxanide, an antiprotozoal agent has shown inhibitory activity against human and animal coronaviruses, at a low-micromolar concentration (Fig. 7.2).

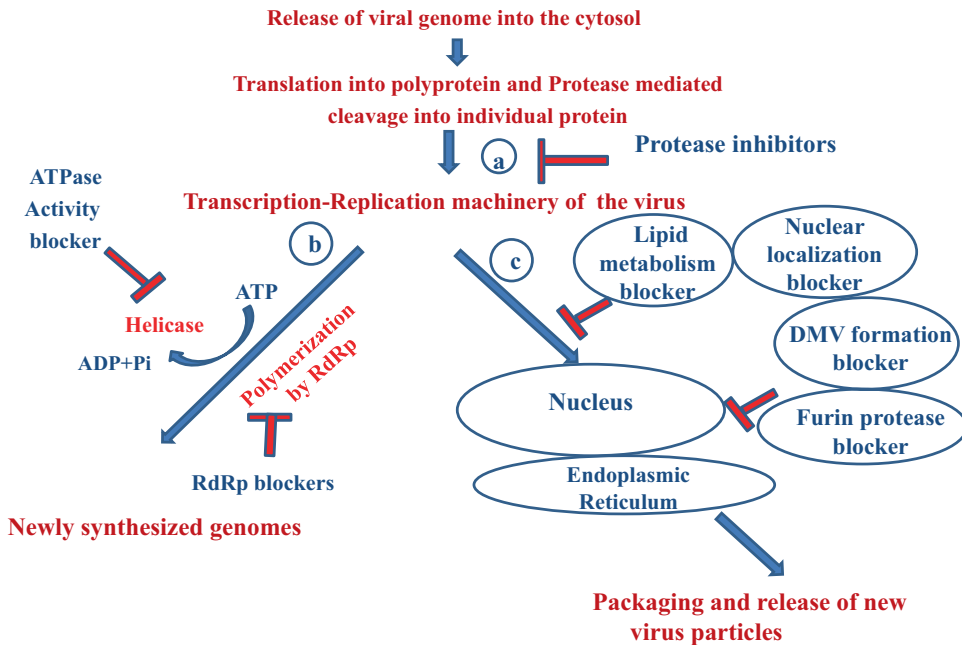


Fig. 7.2 Inhibition of replication cycle of coronavirus inside the host

(a) The viral genome is translated into polyproteins and proteases after entry into the cytosol. The viral main protease cleaves the polyprotein into many non-structural proteins. Inhibition of the main protease can be a way to prevent viral replication inside the host; (b) the transcrip-

tion replication machinery (composed of RdRp, helicase) which produces newly synthesized +/−RNA strands can also be targeted for drug development; (c) other molecular events in the host cell utilized by the coronavirus e.g., lipid metabolism, nuclear localization, transport through double membrane vesicles (DMVs), can also be exploited for drug development

7.10 Inhibition of Viral Helicase

The enzyme helicase, nsp13 has been found to be conserved among different coronaviruses. This enzyme catalyzes NTP-dependent unwinding of the duplex RNA into single strands from 5′ to 3′ direction (Singleton et al. 2007; Jia et al. 2019). The unwinding activity has been postulated for domain 1A (Jia et al. 2019). It has been found that RdRp interacts directly with helicase and increases the overall helicase activity (Jia et al. 2019; Adedeji et al. 2012). Inhibition of the activity of nsp13 can be achieved by targeting NTP binding site or nucleic acid binding site or directly blocking the NTPase activity or helicase translocation, etc. (Habtemariam et al. 2020). Some potential inhibitors have been identified for MERS-CoV helicase based on in silico molecular docking experiments (Zaher et al. 2020). Some of them are benzotriazole, imidazodiazepine, quinoline, anthracycline, triphenylmethane,

pyrrole, small peptide, bananin derivatives (Briguglio et al. 2011), etc. Bananins, an antiviral compound with trioxa adamantane moiety have been shown to inhibit coronavirus helicase activity at a very low concentration (Tanner et al. 2005) (Fig. 7.2).

7.11 Modulation of Host Cell Lipid Metabolism and Inhibition of Viral Double Membrane Vesicle Formations

The lipid metabolism pathway is hijacked by most of the enveloped viruses and biosynthesis of lipid molecules is enhanced to produce viral envelope. An agent that interferes with cholesterol depletion in cells is Methyl-β-cyclodextrin (MβCD). Pretreatment with MβCD has been found to inhibit the production and release of SARS-CoV particles in Vero E6 cells. The nor-

mal viral life cycle was restored by the addition of cholesterol to the culture medium indicating the role of M β CD mediated loss of cholesterol (Li et al. 2007). The interaction between S protein and ACE2 reduces in cells treated with M β CD in a dose-dependent manner leading to reduced viral replication. M β CD treatment successfully inhibited poliovirus entry into in vitro cell culture system (Pranav and Marie 2004).

Similarly, use of phytosterols reduce membrane cholesterol and destabilize the membrane structure and inhibit viral infectivity significantly (Abu-Farha et al. 2020). The cholesterol biosynthesis pathway is reduced by Statins, which inhibit the activity of HMG-CoA reductase. They exert pleiotropic effects on inflammation and oxidative stress, modulate the immune response and restore the vascular redox balance by reducing reactive oxygen species and increasing antioxidants (Castiglione et al. 2020).

Another potential target site is sphingolipid biosynthesis pathway as lipid rafts involved in coronavirus life cycle are enriched in sphingolipids, cholesterol, and many other proteins. Sphingolipids are very important for lungs as they protect the lungs from pulmonary leak and injury. Hence, this pathway can be modulated for therapeutic intervention strategies (Pratelli and Colao 2015).

Double-membrane vesicles (DMVs) formed from ER during the replication of coronavirus can be another target for drugs. DMVs have been found to carry out the replication-transcription complexes or RTCs composed of non-structural proteins 3, 4, and 6 (Angelini et al. 2013). Drugs targeting DMV formation can prevent viral infection by impairing viral RNA synthesis. K22 is one such compound which interacts with nsp6 and inhibits replication of many animal and human coronavirus (Lundin et al. 2014). K22 resistance has been found in nsp6 mutants suggesting the inhibitory role of this molecule. K22 is also active against other viruses like Nidoviruses and Flaviviruses, suggesting its involvement in a critical and conserved step during viral replication (Rappe et al. 2018). Hence, DMVs can be targeted to develop broad-spectrum antivirals and more studies are needed to fully

elucidate their roles in viral life cycle (Shahmohamadnejad et al. 2020) (Fig. 7.2).

7.12 Attenuating the Inflammatory Response

Extensive replication of coronavirus in the epithelial cells and pneumocytes of the respiratory system results in acute inflammation. This process starts when the virus binds to the Toll like receptors (TLR) on macrophages and dendritic cells and unlocks a cascade of signaling pathway leading to activation of IL-1, IL-6, IL-8, tumor necrosis factor- α , and decrease in CD4+ and CD8+ T cells. Other cytokines like IL-2, IL-7, IL-10, G-CSF, IP-10, MCP-1, and MIP-1A have been confirmed at increased levels in many respiratory viral infections, including COVID-19 patients (Huang et al. 2020). The levels of these cytokines correlate with the rate of replication of SARS-CoV-2 (Conti et al. 2020) and lead to cytokine storm. This situation was more visible in elderly patients (over 60 years old) with comorbidities like diabetes, hypertension, etc. (Huang et al. 2005; Chen et al. 2013; Channappanavar and Perlman 2017). Hence, reducing the cytokine storm can be a way of treating the infection. Use of antibodies against inflammatory cytokine IL-6 can be another approach as blocking of the JAK-STAT pathway prevents the cytokine storms in the immune system, one of the reasons behind organ failure and death in many patients. We also have other cytokines such as IL-37 and IL-38 that trigger anti-inflammatory mechanism through diverse mechanisms. For example, IL-37 can increase the levels of AMP kinase and decrease mTOR signaling leading to the inhibition of expression of pro-inflammatory cytokines (Fig. 7.3).

The mTOR or mammalian target of rapamycin, a serine/threonine kinase, is an important signaling protein with crucial functions in cell growth, metabolism, and proliferation (Polivka and Janku, 2014). This protein is also involved in the inflammatory process. Many DNA and RNA viruses utilize this signaling pathway for their replication in mammalian host cells (Cooray

2004; Wang et al. 2006; Shin et al. 2007; Buchkovich et al. 2008; Qin et al. 2011) and inhibitors of mTOR, everolimus and sirolimus, have shown antiviral effects against MERS-CoV (Kindrachuk et al. 2015). In another study, the essential role of mTOR has been shown on antigen-specific humoral immune responses in rapamycin-treated mice (Ye et al. 2017). Furthermore, mTOR inhibitors enhance the production of CD8+ T cells during vaccination (Turner et al. 2011) and could suppress B cell production in germinal centers. Inhibitors of mTOR have been found to successfully prevent severe pneumonia and acute respiratory failure caused by H1N1 (Wang et al. 2014). There were some studies suggesting the positive effect of mTOR inhibitors on early stages of SARS-CoV2 infection, especially in the high-risk groups of patients (Liu et al. 2020; Zheng and Liu 2020; Zhou et al. 2020). However, there are some restrictions regarding the use of mTOR inhibitors along with other drugs. For example, lopinavir/ritonavir used in the treatment of HIV should not be co-administered with sirolimus (rapamycin) and everolimus (Boettler et al. 2020; Guillen et al. 2020). With this limitation in mind, further studies on mTOR inhibitors as a target for SARS-CoV-2 can be done.

In a randomized controlled trial started in March 2020 in UK (RECOVERY), against coronavirus treatment, many potential therapeutic agents were tested. The findings revealed in a press release suggest that Dexamethasone is effective on terminally ill patients who are in ventilator or oxygen support. It showed no effect on the patients with mild symptoms as the steroid acts on and prevents the cytokine storm, the ultimate cause of death, generated in advanced stages in COVID19 patients (Ledford H, 2020). (Fig. 7.3).

7.12.1 Other Strategies

Apart from these above-mentioned strategies, there are more sites in the viral life cycle which can be targeted for drug development.

Coronavirus accessory protein 6 encoded by ORF6 has been found to interfere with nuclear import factors or karyopherin-dependent transcription factors of host cells. ORF6, located in the endoplasmic reticulum of the infected cells, has been found to antagonize many transcription factors like STST1, VDR, CREB1, SMAD4, p53, etc., which are responsible for mediating antiviral responses and other important cellular functions. Thus, blocking ORF6 can reduce the inhibition of antiviral mechanism (Frieman et al. 2007; Hussain et al. 2010). Also, inhibition of nuclear import proteins IMP a/b, which transport proteins to nucleus through nuclear pore complex, can be targeted. One such compound identified is Ivermectin, which acts against SARS-CoV2 in vitro by preventing IMP from binding to viral proteins (Heidary et al. 2020).

Detailed research on the viral life cycle and its interaction with the host immune system will bring out more possible and effective target sites and effective treatment in future.

7.13 Conclusion

Since there is no available treatment for COVID19 at present, palliative care is the only way to relieve some symptoms in the sufferers. Repurposing of some drugs and convalescent plasma therapy, i.e., administration of serum from an individual successfully recovered from the infection, are the two ways of treatment. However, because of the rapid spread of this virus, vaccination seems to be the only efficient method to prevent and control the COVID-19 pandemic. More research should be conducted on coronavirus so that the world remains ready with proper therapeutic interventions in future.

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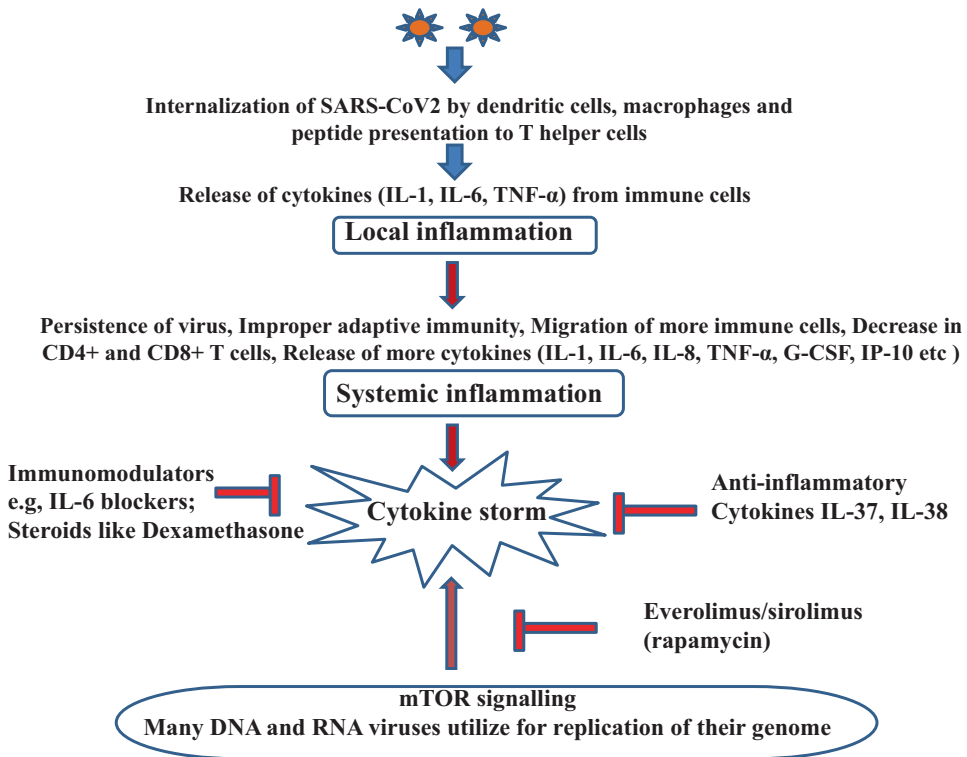


Fig. 7.3 Blocking of cytokine storm can prevent death from COVID19

Cytokine storm, produced by release of excess cytokines and chemokines by immune cells, is the cause of death in

many patients in COVID19. Inhibition of cytokine storm using steroids like dexamethasone (Ledford H, 2020), antibodies against IL6, mTOR blocker like Everolimus have been found to be effective

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