


REVIEW

Interactions between SARS coronavirus 2 papain-like protease and immune system: A potential drug target for the treatment of COVID-19

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Abstract

Coronaviruses (CoVs) are a large family of respiratory viruses which can cause mild to moderate upper respiratory tract infections. Recently, new coronavirus named as Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been identified which is a major threat to public health. Innate immune responses play a vital role in a host's defence against viruses. Interestingly, CoVs have evolved elaborate strategies to evade the complex system of sensors and signalling molecules to suppress host immunity. SARS-CoV-2 papain-like protease (PLpro), as an important coronavirus enzyme, regulates viral spread and innate immune responses. SCoV-2 PLpro is multifunctional enzyme with deubiquitinating (DUB) and deISGylating activity. The PLpro can interact with key regulators in signalling pathways such as STING, NF- κ B, cytokine production, MAPK and TGF- β and hijack those to block the immune responses. Therefore, the PLpro can be as an important target for the treatment of COVID-19. Until now, several drugs or compounds have been identified that can inhibit PLpro activity. Here we discuss about the dysregulation effects of PLpro on immune system and drugs that have potential inhibitors for SCoV-2 PLpro.

1 | INTRODUCTION

The innate immune system is the first line of defence against viral infection and characterized by production of type I interferons (IFN- α and β).¹ Antiviral response relies on pattern-recognition receptors (PRRs) of the innate immune system which recognize pathogen-associated molecular patterns (PAMPs).² The response is initiated by cytoplasmic protein sensors such as RIG-I (retinoic acid-inducible gene I), melanoma differentiation-associated protein 5 (MDA5) and membrane sensors as toll-like receptors (TLR3, 7, 8 and 9).³ Type I IFNs induce the activation of signal transducer and activator of transcription (STAT) factors that induce the expression of hundreds of IFN-stimulated genes (ISGs) which act as antiviral effectors to control viral replication and spread.⁴

Many viruses encode proteins that antagonize both the innate and adapted arms of the immune response.⁵ All CoVs encode at least one papain-like protease (PLpro) with deubiquitinating (DUB), deISGylating (deISG) and other activities that elicit the appropriate innate immune response.⁶ Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) recently entered the human population at the end of 2019 from Hunnan seafood market in Wuhan, China.⁷ The virus causes a pandemic infection in more than 119 million people with a case fatality ratio (CFR) of 1.4% with substantially higher ratios in older age groups, 0.32% in those aged <60 years, 6.4% in those aged \geq 60 years and up to 13.4% in those aged 80 years or older.^{8,9} This raises an urgent need to develop an effective treatment based on identifying targets for viral factors which blocked or reduced innate immune responses. A majority of the newly reported studies showed that PLpro,

which controls replication of the SCoV-2, has been identified as a potential drug target for the treatment.

2 | SARS-CoV-2

The CoVs belongs to the order *Nidovirales*, family of *Coronaviridae*, subfamily *Orthocoronavirinae*, and this subfamily includes *Alphacoronavirus* (α CoV), *Betacoronavirus* (β CoV), *Deltacoronavirus* (δ CoV) and *Gammacoronavirus* (γ CoV).¹⁰ CoVs commonly infect humans and several other vertebrates and can cause acute respiratory distress syndrome (ARDS) with various symptoms such as weakness, fever, breathing difficulty, dry or hacking cough, headaches, pneumonia, enteric, hepatic and neurologic diseases.^{11,12}

Four common human coronaviruses (HCoV-229E, HCoV-HKU1, HCoV-NL63 and HCoV-OC43) cause of mild to moderate common colds.¹³ Before 2003, Human CoVs were known to cause mild respiratory tract infections.¹⁴ In 2003, severe acute respiratory syndrome coronavirus (SARS-CoV, lineage B β CoV) in China and ten years later, Middle East respiratory syndrome coronavirus (MERS-CoV, lineage C β CoV) in Saudi Arabia have caused human epidemics.^{15,16} Both MERS-CoV and SARS-CoV have much higher CFR 34% and 10%, respectively.¹⁷ Recently, a novel flu-like coronavirus (2019-nCoV), named as SCoV-2 (lineage B β CoV) by ICTV Coronaviridae Study Group on February 12, 2020, was found at the end of 2019 in China.¹⁸ As of March 14, 2021, the WHO has reported that there are more than 119 million confirmed cases globally with more than 2.5 million deaths.¹⁹

SCoV-2 has structural similarity to the coronaviruses, more specifically SCoV, and contains a single-stranded and positive-sense RNA genome comprising approximately 29,903 nucleotides that has 5' and 3' terminal sequences containing 12 open reading frames (ORFs) (Figure 1).²⁰

Like other coronaviruses, ORF1a/ORF1b is the biggest and encodes two large overlapping replicase polyproteins 1a (pp1a) and pp1ab. These precursors polyproteins are processed by two viral cysteine proteases (PLpro and 3CLpro) into 16 non-structural proteins (NSP 1-16) (Table 1).²¹⁻²³

SCoV-2 shares more than 79.5% of its genome and protein homology (95%–100%) with SCoV.²⁴ In this respect, Chan et al²² demonstrated no remarkable differences between the ORFs and NSPs of SCoV-2 and SCoV. SCoV encodes seven innate immune antagonists, including nsp1, nsp3, nsp 16, nucleocapsid (N) protein, membrane (M) protein and the products of ORF6 and ORF3b.^{3,25} They share 84, 76, 93, 94, 91, 69 and 32% amino acid identity with their counterparts in SCoV-2 respectively.²⁶ The loss of ORF3b and significant changes in ORF6 can reduce capacity of SCoV-2 to modulate type I IFN responses rather than SCoV whereas PLpro deubiquitinating domain in nsp3 remains intact.⁷ However SCoV

and SCoV-2 have similarities and differences relevant to deubiquitin activity of PLpro.²⁴

A detailed knowledge of how PLpro interacts with the host innate immune system is very important for understanding of the pathogenesis of SCoV-2. In this review, the interactions between PLpro of SCoV-2 and IFN response are described. A map of the pathways that will be discussed in the text is given in Figure 2.

3 | PL^{pro} DOMAIN

After the virion has entered the host cell, pp1a and pp1ab cleaved by two viral proteases, main protease (3CLpro, also called main protease) and PL^{pro}.²⁷ 3CLpro is a dimer which utilizes a Cys/His catalytic dyad, whereas PLpro is a monomer with a Asp/His/Cys canonical catalytic triad.²⁸

Most coronaviruses encode two PLpro, termed PL1pro and PL2pro, whereas SCoV and 2 encode a single PLpro (Table 1).^{23,29} PLpro is the C-terminus of nsp3a and acts as a multifunctional cysteine protease along with phosphatase activity that processes the viral polyprotein and hosts cell proteins via the formation of an isopeptide bond and hydrolyzing the peptide in viral and cellular substrates that participates in viral replication, regulates immune responses and antagonizes interferon (IFN) molecules.^{30,31} SCoV-2 PLpro is made up of an N-terminal ubiquitin-like domain (found in many ubiquitin-specific proteases or USPs) and a C-terminal domain containing thumb and palm, where the catalytic triad is situated, and the fingers, which include the zinc-finger motif.³¹ SCoV-2 PLpro contains 945 nucleotides with 315 amino acid (residue 1564–1878, 35.6 kDa). The Amino acid sequence identity between the PLpro of SCoV-2 with SCoV is 83%. As mentioned above, with this amino acid similarity there are differences in the biochemical characterization of SCoV-2 and SCoV.³²

4 | DUBs AND deISGylating

In addition to protease activity, PLpro is recognized to be involved in deubiquitination and deISG.³³ Post-translational modification (PTMs) of proteins including phosphorylation, methylation, lipidation, acetylation, glycosylation and ubiquitination occurs in almost all proteins that regulate protein function and numerous cellular processes.³⁴ Ubiquitination is common PTMs which involves covalent linkage of 76-residue-long polypeptide (8.5 kDa) ubiquitin molecules (Ub) with seven lysine (K) residues (K6, K11, K27, K29, K33, K48 and K63) to substrate proteins as monomers (monoubiquitination) or polymers (polyubiquitination).^{35,36} Polyubiquitination occurs between the carboxy terminal glycine of a Ub and an internal lysine of another Ub.³⁷

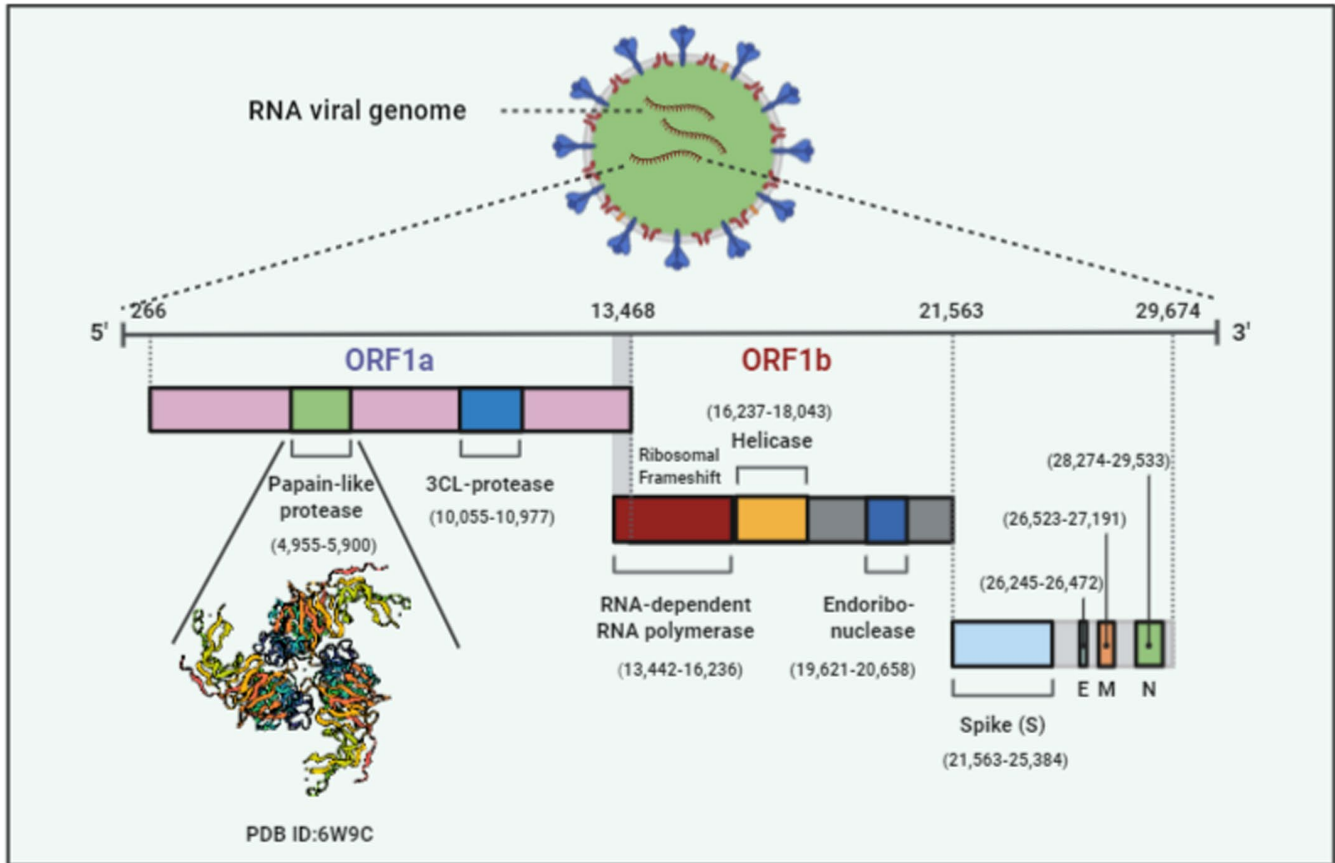


FIGURE 1 Schematic diagram of SARS-CoV-2 genome organization. The single-stranded RNA genome is 29903 nucleotides in size (NC-045512), encoding 9967 amino acids. The G + C content is 37.97%. There are 12 putative, functional ORFs. The large replicase polyproteins 1a (490 kDa) and 1ab (794 kDa) cleaved into 16 putative NSPs. The 5' and 3' UTRs are 265 and 358 nucleotides long, respectively

Predominant linkage type in cells is Lys48-linked chains and their role is to target proteins for proteasomal degradation.³⁸ The ubiquitination reaction is catalysed sequentially by three enzymes: the ubiquitin-activating enzyme (E1), the ubiquitin-conjugating enzyme (E2) and the ubiquitin ligase (E3).³⁹ Protein ubiquitination is a reversible modification regulated by deubiquitinating enzymes (DUBs) and implicated in cell cycle regulation, protein degradation, gene expression, DNA repair, autophagy, etc.⁴⁰⁻⁴²

The DUBs, a large group of cysteine proteases, cleave ubiquitin or ubiquitin-like proteins from proteins and other substrates. The human genome encodes approximately 103 DUBs which divided into six families: ubiquitin-specific proteases (USPs), Josephins and JAB1/MPN/MOV34 metalloenzymes, ubiquitin C-terminal hydrolases (UCHs), motif interacting with Ub-containing novel DUB family (MINDY) and ovarian tumour proteases (OTUs).⁴³

Analysis of purified CoV PLPs and the X-ray crystal structure of SCoV PLpro reveal new information on viral DUB activity.⁴⁴ There is evidence for the structural relationship between SCoV PLpro and ubiquitin C-terminal hydrolase (UCH-L1), ubiquitin-specific protease 14 (USP14) and

herpes-associated ubiquitin-specific protease (HAUSP or USP7).^{45,46} SCoV PLpro with high affinity recognize poly Ub chains by reading units of a Lys48 and remove Lys48 from polyubiquitin chains.^{47,48} Recently studies showed that SCoV-2 PLpro have DUB activity.^{45,49} SCoV-2 PLpro cleaves K48-linked ubiquitin chains at a substantially slower rate than that of SCoV PLpro. Like the SCoV, SCoV-2 PLpro shows no appreciable activity for K63 linked polyubiquitin chains.³²

Sulea et al⁴⁵ showed that PLpro inhibits innate immune responses with interferon-sensitive gene 15 (ISG15) conjugation in a process referred to as deISGylating. ISG15, an antiviral ubiquitin-like protein (Ubl) with two tandem ubiquitin-like folds, is expressed and secreted by human monocytes and lymphocytes and in response to IFN- α and β .^{50,51} ISG15 conjugated with many targets (ISGylation), including Janus tyrosine kinase (JAK), signal transducer and activator of transcription (STAT) and IRF3 proteins and significantly leads to up-regulation following cellular stimulation by IFNs or viral infection.^{48,51,52} Apart from the induction of immune responses, degradation or sequestration of viral proteins via ISGylation has also been found to play a role in host immunity.⁵³

TABLE 1 Classification of Coronaviruses

Viruses	Hosts	No. of nsp in ORF1ab	No. of papain-like proteases
<i>Alphacoronavirus</i>			
Transmissible gastroenteritis virus	Pigs	16	2
Porcine respiratory coronavirus	Pigs	16	2
Feline coronavirus	Cats	16	2
Human coronavirus 229E	Humans	16	2
Human coronavirus NL63	Humans	16	2
Porcine epidemic diarrhoea virus	Pigs	16	2
Scotophilus bat coronavirus 512	Lesser Asiatic yellow house bats	16	2
Rhinolophus bat coronavirus HKU2	Chinese horseshoe bats	16	2
Miniopterus bat coronavirus HKU8	Bent-winged bats	16	2
Miniopterus bat coronavirus 1A	Bent-winged bats	16	2
Miniopterus bat coronavirus 1B	Bent-winged bats	16	2
<i>Betacoronavirus</i>			
Subgroup A			
Human coronavirus OC43	Humans	16	2
Bovine coronavirus	Cows	16	2
Porcine hemagglutinating encephalomyelitis virus	Pigs	16	2
Equine coronavirus	Horses	16	2
Human coronavirus HKU1	Humans	16	2
Mouse hepatitis virus	Mice	16	2
Subgroup B			
SARS coronavirus 1	Humans	16	1
SARS coronavirus 2	Bats, Humans	16	1
SARS-related Rhinolophus bat coronavirus HKU3	Chinese horseshoe bats	16	1
Subgroup C			
Tylonycteris bat coronavirus HKU4	Lesser bamboo bats	16	1
Pipistrellus bat coronavirus HKU5	Japanese pipistrelle bats	16	1
Middle East Respiratory Syndrome	dromedary camels, Humans	16	1
Subgroup D			
Rousettus bat coronavirus HKU9	Leschenault's rousette bats	16	1
<i>Gammacoronavirus</i>			
Infectious bronchitis virus	Chickens	15	1
Turkey coronavirus	Turkeys	15	1
Beluga whale coronavirus	Beluga whales	15	1
<i>Deltacoronavirus</i>			
Bulbul coronavirus HKU11	Chinese bulbuls	15	1
Thrush coronavirus HKU12	Grey-backed thrushes	15	1
Munia coronavirus HKU13	White-rumped munias	15	1

In this context, researchers found ISG15 significantly enriched in complexes with SCoV-2 PLpro compared with SARS-CoV-1 PLpro.^{51,54} Shin et al showed SCoV-2 PLpro and SCoV PLpro in vitro decreased ISGylation including

ISGylation of IRF3, but SCoV-2 PLpro having a more potent effect.⁵⁴ Generation of unconjugated form of ISG15, enhances the secretion and extracellular signalling function of ISG15, which causes production of pro-inflammatory

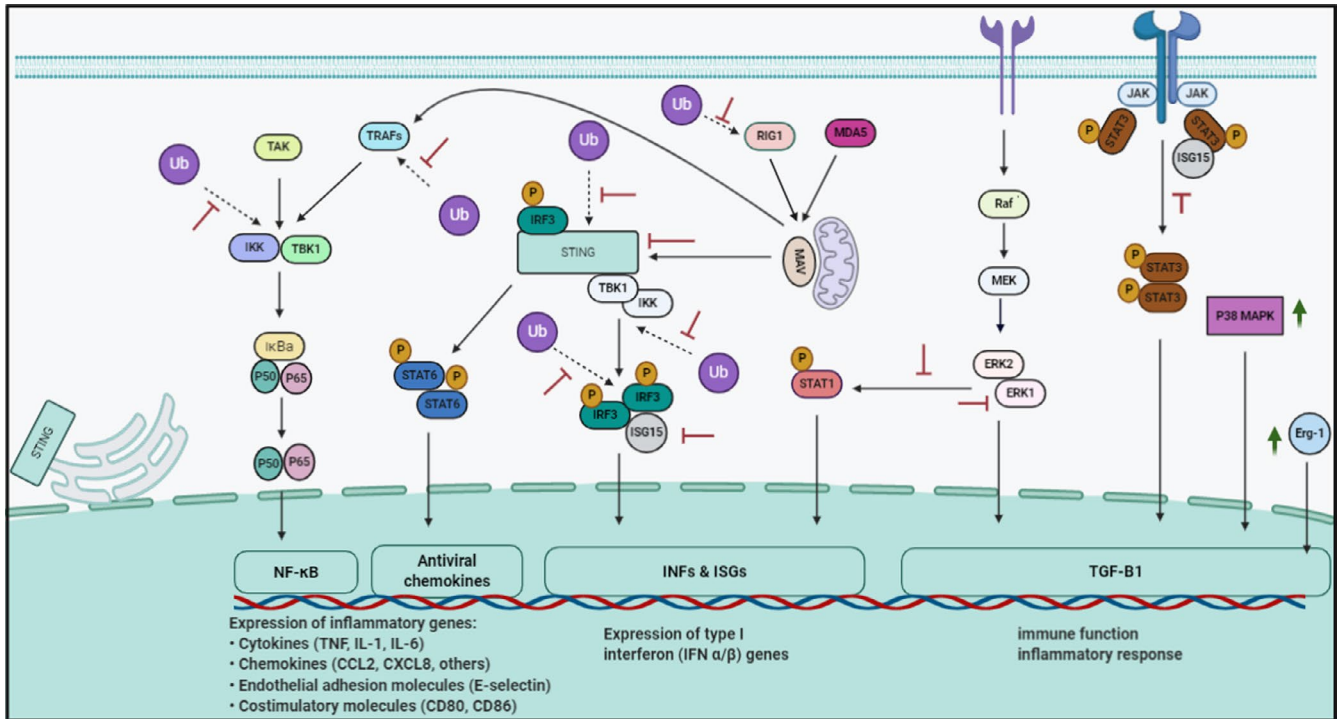


FIGURE 2 Interaction between signalling pathways and PLpro. See text for more details

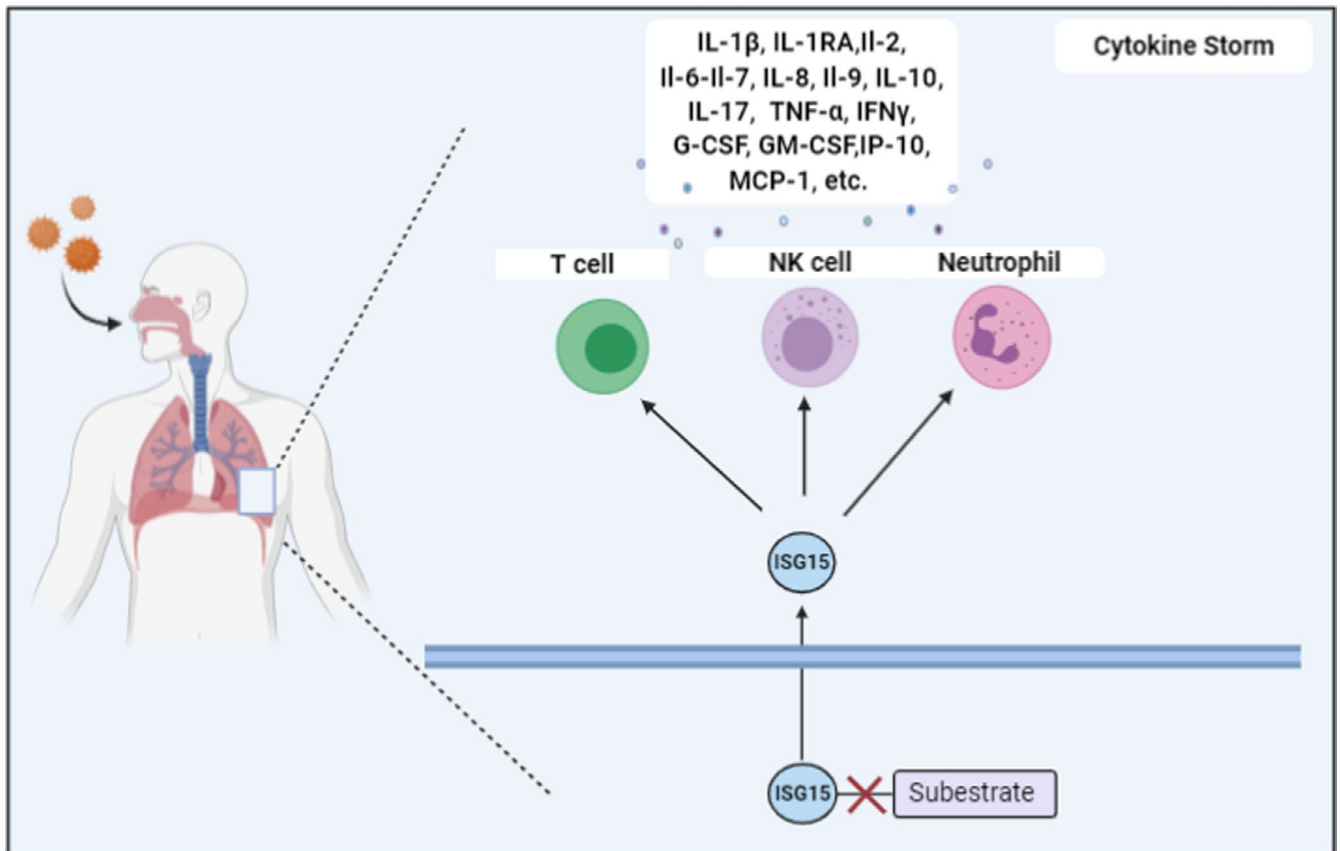


FIGURE 3 ISG15 is conjugated to a wide range of viral and cellular proteins, influencing immune responses. The PLpro can counter ISGylation by deconjugating ISG15. The ISG15 can be secreted from cells and can induce the secretion of inflammatory cytokines

cytokine from cells of the immune system⁵⁵ (Figure 3). Pro-inflammatory cytokines secretion is characterized by 'cytokine storm' which may lead to acute respiratory distress syndrome (ARDS) in severe COVID-19 patients.⁵⁶

Overall, SCoV-2 PLpro have deISGylating and DUB activities to promote the suppression of the innate immune response by effect on IFN and signalling pathways.⁶ The PLpro as a drug candidate can a way to reduce cytokine storms associated with COVID-19.

5 | STING SIGNALLING PATHWAY

STING (stimulator of interferon genes, also known as MITA, ERIS and MPYS) is a key scaffolding protein which be essential for protecting the cell against a variety of pathogens and it could prevent the development of cancer by promoting antitumour immune response.⁵⁷ STING, is an ER-associated membrane protein with four transmembrane domains in the N-terminal region.⁵⁸ Downstream effects of STING activation include Nuclear factor kappa B (NF- κ B), IFN regulatory factor-3 (IRF3) and STAT6 activation, which leads to the production of type I IFNs.⁵⁹

Stimulation induces dimerization and phosphorylation of STING and triggers accumulation of STING complexed with TANK-binding kinase 1 (TBK1) from the ER to endosomal-lysosomal-perinuclear regions. Activated TBK1 leads to phosphorylation of IRF3 and NF- κ B, which translocate to the nucleus and initiate innate immune gene transcription.⁶⁰ Actually, STING is a key scaffolding protein that links the cytosolic viral RNA sensors RIG-I and MDA5 to the mitochondrial antiviral-signalling protein (MAVS).⁶¹ The activation of STING facilitates the recruitment of IRF3 and TBK1 into a complex where IRF3 is phosphorylated.⁶²

SARS PLpro target STING and indirectly prevent from activating IRF3.⁶³ In this respect, Matthews et al suggested SARS PLpro blocks the innate sensing pathway by inhibiting IRF3 activation through binding to STING.⁶⁴ In another study, Frieman et al⁶⁵ identified PLpro blocks the phosphorylation of IRF3 and does not directly bind to IRF3. Sun et al showed PLpro target STING in cells infected with SCoV and reduced STING dimerization. This question that the PLpro-STING interaction is direct or indirect still unclear. In a novel mechanism SARS PLpro reduces the levels of ubiquitinated forms of STING, RIG-I, TBK1, TRAF3 and IRF3, in which suppresses STING-TRAF3-TBK1 signalling pathway, then negatively regulates IRF3 activation.⁶³ This result agreement with Wen li et al study that SARS PLpro decreased the polyubiquitin forms of TRAF3 and TRAF6. Activated of TRAF3 and TRAF6 required for its E3 ubiquitin ligase activity of TRAF3 and TRAF6. Therefore decrease in ubiquitin form of TRAF3 and TRAF6 correlated with lower levels of TBK1 phosphorylation.²¹

6 | NF- κ B SIGNALLING PATHWAY

The NF- κ B signalling pathway plays critical role in regulation of innate and adaptive immunity, inflammation, apoptosis, cancer and tumour development.⁶⁶ NF- κ B is a transcription factor, consists of five related proteins, p105/p50 (NF- κ B1) and p100/p52 (NF- κ B2), p65 (RelA), RelB and c-Rel (Rel), which in resting state remain in the cytoplasm as dimers associated with the I κ B inhibitor.⁶⁷ There are eight I κ B proteins, I κ B α , I κ B β , I κ B ϵ , I κ B ζ , BCL-3, I κ Bns and the precursor proteins NF- κ B2 and NF- κ B1, which are characterized by the presence of six to seven ankyrin repeat motifs (ANK) which have binding ability to NF- κ B dimers.^{68,69} Therefore, in unstimulated cells, NF- κ B dimers bind to I κ B inhibitor proteins in the cytoplasm because all NF- κ B proteins are characterized by the presence of a highly conserved Rel homology domain (RHD) in their N-terminus, which contains a nuclear localization signal (NLS) and is responsible interaction with I κ Bs.⁷⁰ Upon stimulation, I κ B is phosphorylated in serine residues by the I κ B kinase (IKK) complex, which consists of two catalytic subunits, IKK α (IKK1 or CHUK) and IKK β (IKK2) and an NF- κ B essential modifier (NEMO, also known as IKK γ , IKKAP1 or Fip-3).^{71,72} Phosphorylated I κ B creates a destruction motif recognized by the ubiquitin ligase complex and degraded by 26S proteasome, then NF- κ B complexes translocate to the nucleus and regulates the expression of its target genes.^{38,73} Ubiquitination plays a crucial role in control of NF- κ B pathway as a major regulator of the immune response.⁷³ USP15 inhibits the NF- κ B pathway by removing K48-Ub from I κ B α and consequently prevent degradation it. In this respect, Frieman et al demonstrated that SARS PL^{pro} stabilizes the I κ B α and thereby blocks the activation of the NF- κ B pathway.⁶⁵ In another study Ratia et al⁴⁸ indicated PLpro prevents this degradation of I κ B α and leads to an increase in levels of I κ B α .

Also TBK1 phosphorylates the IRF3, thereby no detectable level of IRF3 phosphorylation decrease of NF- κ B p65 phosphorylation.⁷⁴ As mention above, SARS PLpro reduces the levels of ubiquitinated form of TBK1.²¹ These results conclude that SARS PLpro negatively regulates the NF- κ B signal.

7 | MAPK SIGNALLING PATHWAY

The mitogen-activated protein kinase (MAPK), serine/threonine kinases, acts as an important factor in the intracellular signalling network.⁷⁵ MAPKs consist of four distinct groups: The extracellular signal-related kinases (ERKs), the c-jun N-terminal kinases (JNKs), the atypical MAPKs (ERK3, ERK5 and ERK8) and the p38 MAPKs.⁷⁶ ERK pathway plays a crucial role in the regulation of cellular processes. Activation of the ERK pathway includes three signal cascades, Raf, MEK1/2 and ERK1/2. Upon stimulation, Raf

kinase is activated, which then activates the MEK1/2 and subsequently the activated MEK1/2 phosphorylate activate the ERK1/2. Finally, the activated ERK1/2 translocates from cytoplasm to nucleus and phosphorylates a large number of downstream substrates such as transcription factors regulating transcription for a large number of genes.⁷⁷ Therefore, the ERK signalling pathway involves a variety of cellular activities including cell growth, differentiation, survival or apoptosis.⁷⁸ Phosphorylation of STAT1 at serine 727 by ERK1/2 and p38 MAPK facilitates nuclear translocation of STAT1 for full expression of antiviral genes like protein kinase R (PKR), 29-59-oligoadenylate synthetase (OAS) and ISG15. Downregulation of ERK1 was identified with suppression of interaction between ERK1 and STAT1 as type I IFN antagonist function of SCoV PLpro.⁷⁹

8 | TGF- β SIGNALLING PATHWAY

The transforming growth factor- β (TGF- β) superfamily of signalling molecules controls a broad range of cellular processes, including cell differentiation, proliferation, embryonic development and remodelling.⁸⁰ The effects of TGF- β are mediated by three known isoforms of TGF- β (TGF- β 1, 2, 3) via TGF- β type I and II receptors and are transduced through Smad and non-Smad pathways. In patients with SCoV-2 virus infection, death has been caused by uncontrolled inflammatory responses, oedema and fibrosis in the lungs.⁸¹ Fibrosis is one of the most important consequences of TGF- β dysregulation.⁸² SCoV-2 virus infection induces massive activation of the TGF- β in the lungs through neutrophil infiltration into the lungs, dysregulation of the coagulation and fibrinolytic pathways and apoptosis of bronchial epithelial cells, pneumocytes and T lymphocytes.⁸¹ It is interesting to note that SCoV PLpro significantly increased TGF- β 1 mRNA expression and protein production in cell-based assay and in mouse model^{83,84} and in early phase of SCoV infection, TGF- β 1 rises in plasma and lung tissues.⁸⁴

TGF- β stimulation can be activated under MAPK cascade, which represents an important mechanism for non-Smad pathways.⁸⁵ One study has shown that ERK1/2 and p38 MAPK inhibitors (U0126 and SB203580) dramatically decreased and expression of many TGF- β 1-associated genes including HSP27, vimentin, protein disulfide isomerase A3 precursor, retinal dehydrogenase 2, glial fibrillary acidic protein, glutathione transferase omega-1 increased.⁸³ Vimentin is the major component of the type III intermediate filament protein which during virus entry it was observed a direct interaction between vimentin and SCoV spike protein.⁸⁴ PLpro up-regulates activating ubiquitin proteasome of UBE2K and proteasome subunit alpha type 5, and p38MAPK and ERK1/2 signalling via increase of HSP27.⁸³ In li et al study, SCoV PLpro significantly triggered Egr-1 dependent activation of TGF- β 1 promoter via ROS/p38 MAPK/STAT3 pathway.⁸⁴

Given the similarities between of SCoV-2 and SCoV, it could be predicted that PLpro can have positive role in the regulation of the cellular inflammatory and immune responses through TGF- β .

9 | PAPAINE-LIKE PROTEASE INHIBITORS

Viral proteases are an attractive target for antiviral drug development.⁵¹ In this context, a study published in Nature discusses about the role of SARS-CoV-1 and SARS-CoV-2 PLpro to host innate immune response and immune evasion.⁵⁴ Although the PLpro is a potential target for CoVs inhibitors, but no inhibitor approved drug by FDA. Table 2 shows sixteen FDA-approved drugs with good affinity for SARS-CoV-2 PLpro.³¹

Computational methods were used for the development of the inhibitors of SCoV-2 PLpro. Molecular docking indicated a series of drugs that exhibit a high binding affinity to SCoV-2 PLpro. Table 3 shows the binding energy scores along with interaction of compounds over the SCoV-2 PLpro. In Kouznetsova et al⁶² study, Nilotinib was found with best docking energy. Canrong Wu et al⁸⁶ based on the results of bioinformatics analysis demonstrated new compounds for further in vitro and in vivo studies of SCoV-2. This finding showed that a series of antiviral drugs (ribavirin, valganciclovir, thymidine), antibacterial drugs (cefpiramide, sulfasalazine, phenethicillin, lymecycline, demeclocycline, doxycycline, oxytetracycline and tigecycline), antiasthmatic drugs (montelukast, fenoterol and reproterol) may have high binding affinity to PLpro. Delre et al released results of inhibitors of the SCoV-2 PLpro. In this study, dasatinib with the best docking score could efficiently bind to SCoV-2 PLpro.⁸⁷ Dasatinib was also shown to be active against SCoV-2 in a case report study.⁸⁸ It is interesting to know that curcumin, a polyphenol extracted from an East Indian plant *Curcuma longa*, can interact with a cysteine residue of PLpro.⁸⁷ Liu et al reported that curcumin has a protective effect on the lung in case of severe pneumonia caused by SCoV-2, decreasing the expression of proinflammatory cytokines.⁸⁹

In addition, Terconazole and fluspirilene were shown to be active in cell-culture assays for SCoV-2.⁸⁸ Another study has shown chloroquine might has antiviral activity through its inhibition of SCoV-2 PLpro.⁹⁰ GRL-0617 with inhibition of SCoV-2 PLpro can reduce the virus cytopathogenic effect (CPE), viral replication and suppression of host innate immune responses in infected cells.³² Recently, Fu et al showed GRL-0617 ($IC_{50} = 2.1 \mu\text{M}$) blocked the binding of the C-terminal tail of ISG15 with PLpro and can be a promising approach for combating COVID-19.⁹¹ One study showed 6-Thioguanine (6-TG) can be considered as a inhibiting PLpro deISGylation, polyprotein cleavage and viral replication of SCoV-2.⁹² Disulfiram, an FDA-approved drug has

TABLE 2 Sixteen FDA-approved drugs with good affinity for SARS-CoV-2 PLpro

Drug name	Mechanism of Action	Binding affinity
Biltricide	Increases cell membrane permeability to calcium	8 nM-8 μ M
Cinacalcet	Increasing the sensitivity of the calcium sensing receptors	26 nM-3 μ M
Procainamide	Sodium channel blocker	30 nM-3 μ M
Terbinafine	Inhibits the enzyme squalene monooxygenase	33 nM-3 μ M
Pethidine	Acts as a weak agonist of opioid receptors	53 nM-5 μ M
Labetalol	Blocking alpha and beta adrenergic receptors	113 nM-11 μ M
Tetrahydrozoline	Agonist of alpha-1 adrenergic receptors	137 nM-14 μ M
Ticlopidine	Preventing platelets from sticking to each other	160 nM-16 μ M
Ethoheptazine	Not available	163 nM-16 μ M
Formoterol	Relaxing smooth muscle and opening up the airways	716 nM-71 μ M
Amitriptyline	Inhibition of serotonin and norepinephrine transporters	466 nM-46 μ M
Naphazoline	Stimulating alpha adrenergic receptors	697 nM-69 μ M
Levamisole	Acetylcholine receptor agonist	259 nM-26 μ M
Benzympenicillin	Interferes with the synthesis of the bacterial cell wall	718 nM-71 μ M
Chloroquine	Preventing the conversion of heme to hemazoin	858 nM-85 μ M
Chlorothiazide	Inhibiting chloride reabsorption	939 nM-93 μ M

TABLE 3 Binding energy scores along with interaction of compounds over the SCoV-2 PLpro

Compound/drug	Binding Energy (kcal/mol)	Ref	Compound/drug	Binding Energy (kcal/mol)	Ref
Oseltamivir	-121.55	98	4'-O-methylbavachalcone	-42.64	98
Sofosbuvir	-119.44	98	Valganciclovir	-42.21	99
Famciclovir	-85.61	98	Penciclovir	-41.75	99
Isobavachalcone	-84.75	98	Quercetin	40.9	100
Tioguanine	-78.64	98	Valganciclovir	-39.13	99
Elsulfavirine	-76.13	99	Ritonavir	-37.6	99
Chromen	-67.92	98	Montelukast	36.4	99
Merimepodib	-67.51	99	Fostamatinib1	33.5	100
Efavirenz	-66.98	98	Azvadine	-33.1	98
Lopinavir	-61.53	99	Nadid	-32.9	100
Phenformin	56.5	100	Psoralidin	-29.89	98
Maribavir	-53.75	99	Candestran	-28.9	100
Papyriflavonol A	-51.99	98	Valsartan	-28.6	100
Ebselen	-50.99	98	Ribavirin (RBV)	-26.49	98
Faldaprevir	-50.77	99	Bavachinin	-25.59	98
Famciclovir	-47.28	99	Disulfiram	-24.84	98
Corylifol A	-46.78	98	Zanamivir	-24.7	100
Mercaptopurine	-46.61	98	GRL-0617	-24.62	98
Inarigivir	-46.23	99	Aminoethyl	-20.87	98
Remdesivir	-45.15	99	Zanamivir	-19.83	99
GS-6620	-44.73	99	Oxyglutathione	-19.5	100
Nelfinavir	-43.48	99	Darunavir	-8.74	98

TABLE 4 Comparison the activity of PLpro between SCoV-1, SCoV-2 for two Ebselen and Naphthalene

Inhibitor	CoV	Inhibition of (IC50, μ M)			Ref.
		Pro	Ub	ISG15	
Ebselen	SCoV	N.d	8.45 \pm 0.96	N.d	101,102
	SCoV-2	0.578 \pm 0.04	2.02 \pm 1.02	N.d	
Naphthalene inhibitors	SCoV	0.15 \pm 0.01	0.66 \pm 0.08	0.66 \pm 0.08	32,54,103,104
	SCoV-2	2.4 \pm 0.02	0.74 \pm 0.07	1.50 \pm 0.08	

also been identified to be a potential therapeutic target for SARS-CoV-2 infection. Tamburin et al⁹³ showed that symptoms compatible with COVID-19 were significantly less common in patient under disulfiram treatment than control group (not taking disulfiram). Disulfiram is known to be a thiol-reactive compound that can covalently modify cysteine residues and may also act as a zinc ejector.⁹⁴ There are four Zn sites in SARSCoV-2 PLpro that is why disulfiram (as Zn-ejector drug) can be used to disrupt Covid-19 protein structure/function.⁹⁵ It is noteworthy that disulfiram has now entered Phase 2 clinical trial. Primary outcome showed change in plasma inflammatory biomarker levels (eg, IL-6, IL-1b) and viral load at days 5, 15 and 31.⁹⁶

At the end of this topic, we compare the activity of PLpro between SCoV and SCoV-2 for two inhibitors which may contribute to speed up therapeutic development of COVID-19 (Table 4).⁹⁷

10 | CONCLUSION

SARS-CoV-2 is a serious public health threat, and dangerous mutation on its genome is an alarm to search for alternative approaches for inhibiting the spread of this virus during an effective vaccine development. The innate immune response can block via proteins encoding by CoVs, so accurate understanding of its molecular process may result in the identification of therapeutic targets which can be used to reduce replication and pathogenesis. The SCoV and SCoV-2 PLpro enzyme is essential for viral replication and block the activation of type I IFN through; (a) reducing of STING dimerization; (b) disruption of MAVS-STING-RIG-I complex formation; (c) deISGylation of ISG15; (d) dysregulation of TGF- β , MAPK and NF- κ B; (e) deubiquitination of RIG-I, STING, IRF3 and TBK1; (f) Prevention of TBK1 phosphorylation through deubiquitination of TRAF3 and TRAF6.

Based on above mentions, the PLpro is responsible for suppression of host innate immune responses, so further characterization of the SARS-CoV-2 PLpro may provide new targets for antiviral interventions. As an outcome of this study, more investigation regarding the ability of the some mentioned compounds with high binding affinity or energy in blocking the entrance of the PLpro active site and inhibiting

PLpro enzyme activity is highly recommended. In this regard, in vivo and in vitro evaluations for candidate drugs and preparation for clinical trial applications are better to do.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in [repository name] at [URL/DOI], reference number [reference number]. These data were derived from the following resources available in the public domain: [list resources and URLs].

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