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Recent emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transpired into pandemic coronavirus disease 2019 (COVID-19). SARS-CoV-2 has been rapidly transmitted across the globe within a short period of time, with more than 106 million cases and 2.3 million deaths. The continuous rise in worldwide cases of COVID-19, transmission dynamics of SARS-CoV-2 including re-infections and enormous case-fatality rates emphasizes the urgent need of potential preventive and therapeutic measures. The development of effective therapeutic and preventive measures relies on understanding the molecular and cellular mechanism of replication exhibited by SARS-CoV-2. The structure of SARS-CoV-2 is ranging from 90–120 nm that comprises surface viral proteins including spike, envelope, membrane which are attached in host lipid bilayer containing the helical nucleocapsid comprising viral RNA. Spike (S) glycoprotein initiates the attachment of SARS-CoV-2 with a widely expressed cellular receptor angiotensin-converting enzyme 2 (ACE2), and subsequent S glycoprotein priming via serine protease TMPRSS2. Prominently, comprehensive analysis of structural insights into the crucial SARS-CoV-2 proteins may lead us to design effective therapeutics molecules. The present article, emphasizes the molecular and structural perspective of SARS-CoV-2 including mechanistic insights in its replication.

1. Introduction

Unprecedented global emergence of novel coronavirus (nCoV) coined as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in late 2019 in Wuhan, China has led the whole world susceptible towards its infection causing a pandemic Coronavirus disease 2019 (COVID-19) [1]. According to the World Health Organization (WHO), SARS-CoV-2 has infected at least 106 million people worldwide with 2.3 million deaths affecting at least 220 countries, areas and territories [2]. The uninterrupted rise in worldwide cases of COVID-19, transmission dynamics of SARS-CoV-2 including re-infections and enormous case-fatality rates emphasizes the urgent need of effective therapeutic and preventive measures [3]. Coronavirus (CoVs) are a family of positive sense enveloped RNA viruses known to infect a wide range of species including avian, humans, livestock and companion animals. Until 2019, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) were the only two known CoVs which causes a severe respiratory illness in humans [4]. SARS-CoV-2 is the 7th CoV which is known to infect human where SARS-CoV and MERS-CoV, SARS-CoV-2 infection results in severe disease and HKU1, OC43, NL63 and 229E were found to cause mild symptoms [5]. Recent emergence of various newly emerging strains of SARS-CoV-2 specifically UK’s SARS-CoV-2 VOC 202012/01 (B.1.1.7), South Africa’s 501Y.V2 and Welsh 501Y variants, imposing serious concern worldwide. Human transmission of SARS-CoV-2 is primarily exhibited via direct, indirect or close contact with infected secretions including respiratory droplets, saliva generated when an infected individual coughs, sneezes, talks or sings [6]. However, other routes of transmission occur via fomite, blood borne, fecal-oral, mother-to-child, and animal-to-human transmission [7]. SARS-CoV-2 primarily targets the respiratory systems, however, complication in other systems including cardiovascular, neurological and renal also contributes to the mortality [8]. So far, the clinical characteristics are considerably heterogeneous which ranges from asymptomatic to mild, moderate and severe disease forms [9]. SARS-CoV-2 infection is characterized by various immunopathological features including lymphopenia, lymphocyte activation and dysfunction, increased level of neutrophils, cytokine storm, depletion and exhaustion of lymphocytes [10]. Still lack of specific treatment for COVID-19 is the major cause of mortality since the treatment mainly depends on supportive care of the patients. Interestingly, a number of therapeutic molecules including repurposed drugs are found efficient for the
treatment of COVID-19 [11–13]. Although a large number of studies and clinical trials are being performed [14,15], a comprehensive understanding of SARS-CoV-2 structure and replication is decisive for designing effective preventive and therapeutic measures.

2. SARS-CoV-2 morphology

Vero cells infected with SARS-CoV-2 subjected to transmission electron microscopy using ultrathin sections have allowed a morphological understanding of the structure of SARS-CoV-2. The tomograms generated by electron microscopy revealed that SARS-CoV-2 is of oval shape. The SARS-CoV-2 and SARS-CoV are not only phylogenetically related but are also similar morphologically. The negative staining of electron microscopy of SARS-CoV-2 revealed a diameter of 90–120 nm which is identical to that of SARS-CoV. In addition, the size of spike glycoprotein is 25 nm [16]. The host membrane-derived lipid bilayer of SARS-CoV-2 incorporates structural proteins encapsulating viral RNA. The genome size of SARS-CoV-2 is ranging from 27 to 32 kb which comprises of 6–11 open reading frames (ORFs) where six functional ORFs are arranged from 5′ to 3′ as replicase (ORF1a/ORF1b), spike (S), envelope (E), membrane (M) and nucleocapsid (N) encodes for structural proteins as well as seven putative ORFs arranged between the structural genes and encodes for accessory proteins [17]. The replicase gene covers 67% of the 5′ genome which encodes for a large polyprotein (pp1ab) that gets proteolytically processed into 16 non-structural proteins (nsps). The nsps comprises various viral cysteine proteases namely as chymotrypsin-like, papain-like protease (nsp3), 3C-like, or main protease (nsp5), RNA binding protein (nsp9), RNA-dependent RNA polymerase (nsp12), helicase (nsp13) and others involved in the replication and transcription of SARS-CoV-2. Moreover, structural proteins and nsps derived from the processed polyprotein, SARS-CoV-2 encode for nine accessory factors from sub-genomic ORFs (3, 3a, 6, 7a, 7b, 8, 9b, 9c and 10) [18] (Fig. 1).

3. Mechanisms of SARS-CoV replication

SARS-CoV-2 infection initiates upon attachment of spike glycoprotein with the ACE2 receptor and priming of S glycoprotein via host cell serine protease TMPRSS2 [19]. Following the entry of SARS-CoV-2, viral RNA is released and immediately undergoes a translation process to generate ORF1a and ORF1b [20]. These polyproteins are processed into nsps which forms viral transcription and replication processes. During the synthesis of nsps, organelles undergoing viral replication form distinctive perinuclear double-membrane vesicles (DMVs), convoluted membranes (CMs) and small open double-membrane spherules (DMSs) that all together creates a defensive microenvironment for transcription of subgenomic (sg mRNAs) and viral RNA replication [21]. The structural proteins get transported into the endoplasmic reticulum (ER) membranes following transportation to Golgi apparatus through ER-to-Golgi intermediate compartment (ERGIC) [22]. Finally, SARS-CoV-2 progeny virions get released from the infected cell by the process of exocytosis [17] (Fig. 2).

4. SARS-CoV-2 spike glycoprotein structure

Spike glycoprotein (S-glycoprotein) is a trimeric fusion protein of class I type that exists in a metastable prefusion conformation of club-like shape of ~20 nm in length (Fig. 3A) [23]. S-glycoprotein
mediates the attachment and entry into the host cells through widely expressed cellular receptor angiotensin-converting enzyme 2 (ACE2) [24]. S-glycoprotein consists of two functional subunits S1 and S2 where S1 subunit comprises the N-terminal domain (NTD), signal sequence and receptor binding domain (RBD) whereas, S2 subunit is involved consists of heptad-repeat (HR) regions as HR-N and HR-C crucial for the generation of coiled coil structure inside the protein ectodomain [24,25]. Several of the cleavage sites including furin, protease and S2′ protease are present at the border of S1 and S2 [26]. The S1 subunit involve in the in the attachment to the peptidase domain (PD) of the ACE2 receptor via RBD and S2 subunit involved in the cell and viral membrane fusion. The processing of S-glycoprotein occurs at the boundary of S1 and S2 subunits that is non-covalently associated in prefusion conformation [23]. The ectodomain consists of a head which is connected to the membrane a slender stalk [27]. The three RBDs are conformationally variable which are shielded by the N-terminal domains (NTDs). Upon binding to the ACE2 receptor, the RBD undergoes hinge-like conformational arrangements that temporarily exposes or hide the binding receptors [28]. These two states are denoted as down i.e. receptor-inaccessible state or up conformation i.e. receptor-accessible state [29].

5. Interaction of RBD and ACE2

The interaction between RBD and ACE2 receptor was found to be similar to the polar interactions observed in SARS-CoV [30]. ACE2 is a type I membrane protein expressed in various tissues including lungs, kidney, heart and intestine [31]. ACE2 protein is a transmembrane protein comprised of N-terminal PD and C-terminal collectrin-like domains (CLD). The X-ray crystallographic structure of RBD-ACE2 complex of SARS-CoV-2 revealed that each PD domain interacts with one RBD where an extended loop region of the RBD interacts by forming a bridge spanning the arch shape α1 helix of the ACE2-PD [32]. Whereas, only limited contributions were observed from the α2 helix and β3 and β4 antiparallel strands. The interaction of RBD with PD can be categorized in 3 clusters where the two edges of the bridge interacts with N and C terminal of α1 helix including few areas of α2 helix and β3 and β4. The crucial interacting residues involved are Gln 498, Thr 500, and Asn 501 from the N-termini of the RBD with Tyr 41, Gln 42, Lys 353, and Arg 357 from ACE2, respectively. The middle of the bridge involves interactions of Lys 417 and Tyr 453 from the RBD with Asp 30 and His 34 of ACE2, respectively. Whereas, the C termini of α1 helix involves interactions of Gln 474 and Phe 486 of the RBD interacting with Gln 24 and Met 82 of ACE2 via H-bonding and van der Waals forces respectively (Fig. 3B) [33]. Interestingly, structure-guided sequence alignment has revealed that SARS-CoV-2 and SARS-CoV RBDs use eight identical interacting residues for attachment to the ACE2 receptor.

6. Structure of main protease (Mpro, also called 3CLpro)

The Mpro acts on at least 11 Leu-Gln ↓ (Ser, Ala, Gly) cleavage sites present on the pp1ab (↓ marks the cleavage site) [34]. Interestingly, Mpro doesn’t share these cleave sites with any human encoded proteases, with the result that Mpro has been found as one of the best-characterized drug targets for combating CoVs [35]. The structure of
SARS-CoV-2 Mpro was found to be similar as SARS-CoV Mpro due to 96% sequence identity and was found to structurally diverge by only 0.53 Å. Mpro is characterized as six-stranded antiparallel β barrels which exhibit the substrate-binding site. The structure of Mpro resembles that of chymotrypsin (residues 10 to 99) as well as picorna 3C protease-like domains = I and II (residues 100 to 182). Whereas, the domain III (residues 198 to 303) forms five helices of globular shape which involve in the dimerization of Mpro exhibited via salt bridge interaction among Arg 4 of one protomer with Glu 290 residue of the other (Fig. 3C) [36]. Dimerization of Mpro has been shown to be crucial for its catalytic activity due to NH2-terminal residues (N-finger) of all protomers interacting with Glu 166 required for substrate specific binding site, S1 pocket of the enzyme.

7. Structure of nucleocapsid protein RNA binding domain

The nucleocapsid (N) protein of coronavirus is a multi-faceted RNA-binding protein crucial for replication and transcription of SARS-CoV-2 [37]. The primary role of N protein is binds to SARS-CoV-2 RNA and form the ribonucleoprotein (RNP) complex. Crystal structure of 47–173 residues of N protein of SARS-CoV-2 revealed that SARS-CoV-2 N-NTD packed into an orthorhombic crystal form while the interaction between the interfaces occurs via residues of β-hairpin fingers and palm regions. The core of the protein is made up of five antiparallel β-strands where a single short helix exists just ahead of β2 strand and protruding β-hairpin between strands β2 and β5 (Fig. 3D) [38].

8. Structure of nsp10/nsp16 2'-O-methylase

Among the nsps, nsp10 and nsp 16 have been shown to form a protein complex that requires for methylation at ribose 2'-O position of intermediate nucleotide of SARS-CoV-2 RNA cap [39]. This methylation is crucial for the conversion of cap-0 to cap-1 viral RNA to mimic the cellular mRNAs for translation and a host defense mechanism. The crystal structure of the protein complex was solved at 2.5 Å resolutions. The two complex proteins were found similar to a RMSD of ~0.2 Å. Altogether, the nsp10/nsp16 2'-O-methylase was found as nsp16 monomer sitting on top of nsp10 monomer where the nsp16 is consists of seven α-helices, twelve β-strands and five 3\(\alpha\) helices. These secondary structures assemble together to form a core comprised of helices αZ, αA, αD, and αE and strands β1–β7. Whereas, bottom of the nsp10 consists of three α-helices, three β-strands and two 3\(\alpha\) helices where two zinc-fingers have been found to stabilizes the structure (Fig. 3E). Within the heterodimer complex, nsp10 is crucial due to its role as a co-factor for nsp16 methylase [39].

9. Conclusions

Although, some studies and clinical trials have been performed with SARS-CoV-2, further studies are still required for designing effective preventive and therapeutic measures. The SARS-CoV-2 and SARS-CoV are not only phylogenetically related but are morphologically similar. Among the structural proteins, the spike which is a trimeric glycoprotein has only been studied extensively for a short period of time although it is
essential for the attachment to the cellular receptor ACE2. In addition to spike, several of the proteins including Mpro, nucleocapsid protein RNA binding domain and nsp10/nsp16 2’-O-methylase are crucial targets for developing antiviral drugs.

10. Future perspectives

The continuous rise in the COVID-19 cases and its severity emphasizes the urgent need of effective preventive and therapeutic measures which crucially depends upon the comprehensive understanding of SARS-CoV-2 structures and replication. Structural perspectives of SARS-CoV-2 biology therefore play a vital role in both identification of effective antiviral therapy and structure based antigenic determinants for potential vaccine candidates. So far, the crucial structures of SARS-CoV-2 have been resolved in a very short span of time which made it possible to design potential effective therapies and a few vaccines. The current vaccines being developed are mostly based on the spike glycoprotein which should be further investigated with the other potential SARS-CoV-2 proteins.

Declaration of Competing Interest

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Author contributions

SKS conceived the idea. S.K. and S.K.S. collected the data, devised the initial draft and reviewed the final draft. S.K.S. and S.K.S. finalized the draft for submission. All authors read and approved the final version of the manuscript.

References

[1] C. Wang, P.W. Horby, F.G. Hayden, G.P. Gao, A novel coronavirus outbreak of global health concern, Lancet (London, England) 395 (10223) (2020) 470–473, https://doi.org/10.1016/S0140-6736(20)30185-9.
[2] Coronavirus disease (COVID-19) pandemic. World Health Organization (WHO). https://www.who.int/emergencies/diseases/novel-coronavirus-2019 (Accessed on 02 Feb 2021).
[3] C. Liu, Q. Zhou, Y. Li, L.V. Garner, S.P. Watkins, L.J. Carter, J. Smoot, A.C. Gregg, C. Liu, Q. Zhou, Y. Li, L.V. Garner, S.P. Watkins, L.J. Carter, J. Smoot, A.C. Gregg, A.D. Daniels, S. Jervey, D. Albaiu, Research and development on therapeutic agents for potential vaccine candidates. So far, the crucial structures of SARS-CoV-2 have been resolved in a very short span of time which made it possible to design potential effective therapies and a few vaccines. The current vaccines being developed are mostly based on the spike glycoprotein which should be further investigated with the other potential SARS-CoV-2 proteins.

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References

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[2] Coronavirus disease (COVID-19) pandemic. World Health Organization (WHO). https://www.who.int/emergencies/diseases/novel-coronavirus-2019 (Accessed on 02 Feb 2021).
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References

[1] C. Wang, P.W. Horby, F.G. Hayden, G.P. Gao, A novel coronavirus outbreak of global health concern, Lancet (London, England) 395 (10223) (2020) 470–473, https://doi.org/10.1016/S0140-6736(20)30185-9.
[2] Coronavirus disease (COVID-19) pandemic. World Health Organization (WHO). https://www.who.int/emergencies/diseases/novel-coronavirus-2019 (Accessed on 02 Feb 2021).
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[30] J. Shang, G. Ye, K. Shi, Y. Wan, C. Luo, H. Aihara, Q. Geng, A. Auerbach, F. Li, Structural basis of receptor recognition by SARS-CoV-2, Nature 581 (7807) (2020) 221–224, https://doi.org/10.1038/s41586-020-2179-y.

[31] J. Lan, J. Ge, J. Yu, S. Shan, H. Zhou, S. Fan, Q. Zhang, X. Shi, Q. Wang, L. Zhang, X. Wang, Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor, Nature 581 (7807) (2020) 215–220, https://doi.org/10.1038/s41586-020-2180-5.

[32] I. Hamming, W. Timens, M.L.C. Bulthuis, A.T. Lely, G.J. Navis, H. van Goor, Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis, J. Pathol. 203 (2) (2004) 631–637, https://doi.org/10.1002/path.v203:210.1002/path.1570.

[33] R. Yan, Y. Zhang, Y. Li, L. Xia, Y. Gao, Q. Zhou, Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2, Science (New York, N.Y.) 367 (6485) (2020) 1444–1448, https://doi.org/10.1126/science.abb2762.

[34] W. Rut, K. Groborz, L. Zhang, X. Sun, M. Zmudzinski, B. Pawlik, X. Wang, D. Jochmans, J. Nefs, W. Młynarski, R. Hilgenfeld, M. Drag, SARS-CoV-2 Mpro inhibitors and activity-based probes for patient-sample imaging, Nat. Chem. Biol. 17 (2) (2021) 222–228, https://doi.org/10.1038/s41589-020-00689-z.

[35] Y. Chen, G. Wang, L. Ouyang, Promising inhibitors targeting Mpro: an ideal strategy for anti-SARS-CoV-2 drug discovery, Signal Transd. Targeted Therapy 5 (1) (2020) 173, https://doi.org/10.1038/s41392-020-00291-8.

[36] L. Zhang, D. Lin, X. Sun, U. Cuth, C. Drosten, L. Sauerhering, S. Becker, K. Rox, R. Hilgenfeld, Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors, Science (New York, N.Y.) 368 (6489) (2020) 409–412, https://doi.org/10.1126/science.abb4955.

[37] M.H. Verheij, M.C. Hagemeijer, M. Ulasli, F. Reggiori, P.J. Rottier, P.S. Masters, C. A. de Haan, The coronavirus nucleocapsid protein is dynamically associated with the replication-transcription complexes, J. Virol. 84 (21) (2010) 11575–11579, https://doi.org/10.1128/JVI.00569-10.

[38] S. Kang, M. Yang, Z. Hong, L. Zhang, Z. Huang, X. Chen, S. He, Z. Zhou, Z. Zhou, Q. Chen, Y. Yan, C. Zhang, H. Shang, S. Chen, Crystal structure of SARS-CoV-2 nucleocapsid protein RNA binding domain reveals potential unique drug targeting sites, Acta Pharm. Sinica B 10 (7) (2020) 1228–1238, https://doi.org/10.1016/j.apsb.2020.04.009.

[39] P. Krafcikova, J. Silhan, R. Nencka, E. Boura, Structural analysis of the SARS-CoV-2 methyltransferase complex involved in RNA cap creation bound to sinefungin, Nat. Commun. 11 (1) (2020) 3717, https://doi.org/10.1038/s41467-020-17495-0.