POSTĘPY MIKROBIOLOGII – ADVANCEMENTS OF MICROBIOLOGY
2020, 59, 3, 197–206
DOI: 10.21307/PM-2020.59.3.14

SARS-COV-2 AND *BETACORONAVIRUS*: WHAT HAVE WE LEARNED IN 8 MONTHS?

Agnieszka Kwiatek*, Monika Adamczyk-Popławska

University of Warsaw, Faculty of Biology, Institute of Microbiology, Department of Molecular Virology

Received in June, accepted in August 2020

Abstract: In 2019, a new human pandemic coronavirus (SARS-CoV-2) emerged in Wuhan, China. We present the knowledge about SARS-CoV-2 compared to SARS-CoV and MERS-CoV. The SARS-CoV-2 is similar to other coronaviruses, nevertheless, differences were observed. Cell entry of SARS-CoV-2 is facilitated by cleavage of spike protein by furin. The receptor-binding motif of SARS-CoV-2 spike protein forms a larger binding interface and more contacts with host receptor ACE2 compared those of in SARS-CoV. Unlike other coronaviruses, the SARS-CoV-2 spike protein has a motif, known to bind integrins. Nucleocapsid protein and RNA-dependent RNA polymerase of SARS-CoV-2 display some structural differences compared to those of SARS-CoV as well. These features may increase the efficiency of the spread of SARS-CoV-2 and indicate the putative targets for specific antiviral therapy.

**SARS-COV-2 I *BETAKORONAWIRUS*:
CZEGO NAUCZYLIŚMY SIĘ W CIĄGU OSTATNICH 8 MIESIĘCY?**

Streszczenie: W 2019 r. w WCuhan w Chinach pojawił się nowy ludzki koronawirus (SARS-CoV-2) wywołując pandemię. W niniejszej pracy przedstawiamy aktualny stan wiedzy o SARS-CoV-2 w odniesieniu do SARS-CoV i MERS-CoV. Pomimo ogólnego podobieństwa zaobserwowano różnice pomiędzy SARS-CoV-2 a SARS-CoV i MERS-CoV. Wniknięcie SARS-CoV-2 do komórki jest ułatwione przez rozszczepienie białka S przez furynę. Powierzchnia oddziaływania białka S SARS-CoV-2 z receptorem ACE2 jest większa i te oddziaływania są silniejsze. W odróżnieniu od innych koronawirusów, białko S SARS-CoV-2 ma motyw wiążący integryny. Białko nukleokapsydu i zależna od RNA polimeraza RNA z SARS-CoV-2 wykazują także różnice strukturalne w porównaniu z tymi z SARS-CoV. Cechy te mogą przyczyniać się do szerokiego rozprzestrzeniania się wirusa oraz wskazują potencjalne cele dla specyficznej terapii przeciwwirusowej.

Key words: SARS-CoV-2, betacoronavirus proteins, spike protein, furin-cleavage site, pathogenesis
Słowa kluczowe: SARS-CoV-2, białka Betakoronawirusów, białko S, furyna, patogeneza

1. Taxonomy of *Coronaviridae*

Coronaviridae family belongs to the order *Nidovirales* and the suborder *Coronavirinae*. The family is divided into two subfamilies: *Orthocoronavirinae* and *Letovirinae*. Viruses infecting humans belong only to *Orthocoronavirinae*, which includes the *Alphacoronavirus* and *Betacoronavirus* genera. Non-human-infecting viruses have been classified in *Gammacoronavirus* and *Deltacoronavirus* – two other genera of *Orthocoronavirinae*

Human coronaviruses 229E (HCoV-229E) and NL63 (HCoV-NL63) represent the genus *Alphacoronavirus*. Severe acute respiratory syndrome-related coronavirus 1 (SARS-CoV), Middle East respiratory syndrome-related coronavirus (MERS-CoV), severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), HCoV-HKU-1, and HCoV-OC43 are members of the genus *Betacoronavirus.* All coronaviruses infecting humans have a zoonotic origin, and bats are most likely the natural hosts for all presently known human CoVs, including SARS-CoV and MERS-CoV viruses [45]. The exact origin of SARS-CoV-2 remains elusive, but *in silico* analysis suggests cross-species transmission [7].

SARS-CoV, MERS-CoV, and SARS-CoV-2 are highly pathogenic, while other human-infecting coronaviruses mainly cause mild, asymptomatic infections.

* Corresponding author: Agnieszka Kwiatek, University of Warsaw, Institute of Microbiology, Department of Molecular Virology, Miecznikowa 1, 02-096 Warsaw, Poland; Phone: 48-22 5541419; Fax: 48-22 5541402; e-mail: akwiat@biol.uw.edu.pl

SARS-CoV emerged first in Guangdong, China in 2002. Between 2002 and 2004 were 2 self-limiting SARS-CoV outbreaks, which resulted in a contagious and potentially life-threatening form of pneumonia. MERS-CoV emerged in the Arabian Peninsula in 2012. The largest known outbreak of MERS outside the Arabian Peninsula occurred in the Republic of Korea in 2015. SARS-CoV-2, which is the etiological factor of coronavirus disease 2019 (COVID-19), was discovered in December 2019 in Wuhan, China. This virus has spread globally causing a pandemic with more than 28 637 952 cases and 917 417 deaths as of 13 September 2020.

2. Structure of *Betacoronavirus* virion

Betacoronavirus are enveloped, round or oval-shaped viruses with a diameter of 60 to 140 nm. The envelope is covered by the spike glycoprotein trimmer (S), anchored in a phospholipid bilayer, giving the appearance of the solar corona, prompting the name, coronaviruses [32]. Among S proteins, the membrane (M) proteins and the envelope (E) proteins are also present in the viral envelope. Virus particles contain helically symmetrical nucleocapsids, composed of one segment of genomic RNA associated with phosphorylated nucleocapsid (N) proteins.

3. Genome of *Betacoronavirus*

The genome of *Betacoronavirus* is ~ 30 kb size, positive-sense, single-stranded RNA. It contains a 5' cap structure along with a 3' poly (A) tail, allowing it to act as an mRNA for translation of polyproteins. The coding sequence is flanked by 5' and 3' untranslated regions (UTRs). The genome may be divided into two parts: the 5' proximal part comprises ORF1a and ORF1b encoding orf1ab polyproteins and represents more than two-thirds of the genome. The 3' part (one-third of the genome) contains genes encoding for structural proteins, including mentioned above S, E, M, and N proteins.

Strain isolates of SARS-CoV-2 have been sequenced (NCBI Virus repository: (https://www.ncbi.nlm.nih.gov/labs/virus). The homology among all SARS-CoV-2 strains is generally high, 99.91%–100% at the nucleotide level and overall genome variation is low. However, variation sites in 1a, 1b, S, 3a, M, 8, and N regions were identified. The most variable positions are 28144 nt in ORF 8 (mutation rate 30.53%), 8782 nt in ORF 1ab (29.47%), and 29095 nt in N gene. Additionally, in spike S1 and orf8 encoding regions, variable regions were shown to be the recombination hot spots [70].

The genome organization and nucleotide sequences of UTRs and ORFs of SARS-CoV-2 and those of SARS-CoV share a high similarity. The SARS-CoV-2 UTRs (265 nt at the 5' terminal and 229 nt at the 3' terminal region), shown ≥ 83.6% nucleotide identities with UTRs of other *Betacoronavirus*. The major differences between SARS-CoV-2 and SARS-CoV at the nucleotide sequence level are found in orf3b, spike and orf8 encoding genes. Differences between SARS-CoV-2 and SARS-CoV and some SARS-CoV-2 variability might affect the rapid evolution, the severity of symptoms and spread of the SARS-CoV-2 [25, 50, 62, 69].

4. Proteins of *Betacoronavirus*

The spike (S) protein monomer is ~ 1200 amino acid long and composed of two subunits S1 and S2. S forms homotrimers protruding from the viral surface. The SARS-CoV-2 S glycoprotein shares 76% amino acid sequence identity with spike protein of human SARS-CoV Urbani and 80% identity with bat SARSr-CoV ZXC21 and ZC45 [12].

Similar to spike protein of SARS-CoV, S glycoprotein of SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) cell surface receptor to mediate the entry of the virus into human host cells [12, 39].

During infection of host cells by SARS-CoV-2, the S glycoprotein, organized in trimers, is activated by proteolytic cleavage performed by host cell proteases at S1/S2 and S2' sites into S1 and S2 subunits. S1/S2 cleavage site includes S1 and S2 sites (Fig. 1).

The S1 site of S1/S2 cleavage site of SARS-CoV-2 spike protein includes several arginine residues (SP**R**RA**R**↓SVAS), which indicates high cleavability [39]. Site 2 of SARS-CoV-2, similar to those in the spike protein of SARS-CoV contain IAY↓TMS [35]. Whereas S2' site of SARS-CoV-2 consists of SKPSK**R**↓SF, and SARS-CoV LKPTK**R**↓SF [35].

N-terminal S1 subunit recognizes ACE2 receptor by a conserved receptor-binding domain (RBD), including receptor binding motif (RBM). The overall structure of the interface between ACE2 and RBM is similar for spike proteins of SARS-CoV1 and SARS-CoV-2 [56, 73]. Both RBMs form a gently concave surface with a ridge on the one side. It binds to the exposed outer surface of the claw-like structure of ACE2. However, compared to RBM of SARS-CoV, RBM of SARS-CoV-2 forms a larger binding interface and more contacts with ACE2, and ACE2-binding ridge in RBM of SARS-CoV-2 has a more compact conformation [73].

Among 14 amino acid positions involved in the interaction of RBM with ACE2 and shared by both spike proteins of SARS-CoV and SARS-CoV-2, 8 are identical and most of the remaining residues have similar biochemical properties [12, 39]. Outside of RBM of spike protein of SARS-CoV-2, there is a unique

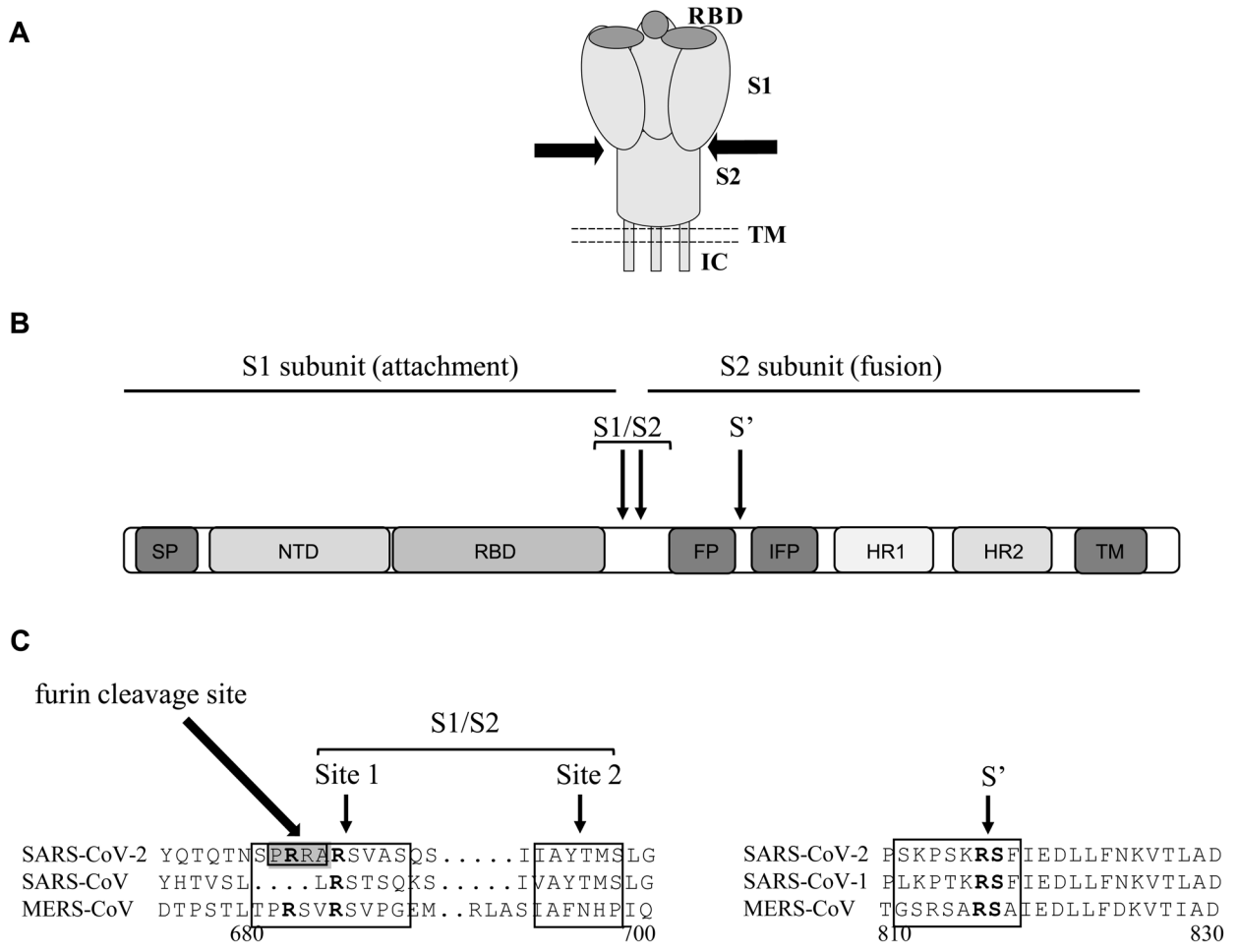

Fig. 1. Scheme illustrating the spike protein of SARS-CoV-2

(A) Scheme of the three-dimensional structure. S1 – S1 subunit, S2 – S2 subunit, RBD – receptor binding domain, TM – transmembrane domain, IC – intracellular tail. The arrows indicate the cleavage site between S1 and S2 subunits; (B) Organization of SARS-CoV-2 spike protein. SP- signal peptide, NTD – N-terminal domain, RBD – receptor binding domain, FP – fusion peptide, IFP – internal fusion peptide, HR1/HR2 – heptad repeat 1/2, TM – transmembrane domain. Arrows indicate the cleavage site S1/S2 and S'; (C) Sequence comparison of the spike proteins of SARS-CoV-2, SARS-CoV and MERS-CoV in a region at S1/S2 boundary. Thin arrows indicate the cleavage site S1/S2 and S', and wide – the furin cleavage site. The cleavage sites are surrounded by frames. Additionally, site cleaved by furin is grey highlighted. Below the amino acids positions are given.

ACE2-interacting residue (Lys417), which forms a salt-bridge interaction with Asp30 of ACE2 [35]. These subtle differences between amino acid sequences of spike proteins may influence the binding affinity of RBDs to ACE2. However, data on the efficiency of binding ACE2 by spike protein of SARS-CoV-2 compared to the efficiency of binding ACE2 by spike protein of SARS-CoV are ambiguous. Walls *et al.* (2020) and Lan *et al.* (2020) demonstrated that the affinity of binding of SARS-CoV-2 spike protein to ACE2 is similar to this of S glycoprotein of SARS-CoV with ACE2 [69, 35, 75]. Whereas, according to Wrapp *et al.* (2020) and Shang *et al.* (2020), RBD of SARS-CoV-2 spike protein has higher efficiency of binding to ACE2 compared to RBD of SARS-CoV spike protein [55, 73].

The more detailed analysis demonstrated that RBD of the spike protein of SARS-CoV-2 is recognized by the extracellular peptidase domain of ACE2 mainly through polar residues [72].

C-terminal, membrane-anchored subunit S2 of spike glycoprotein is responsible for fusion of viral envelope with the cellular membrane. The fusion peptide (FP), a second proteolytic site (S2'), an internal fusion peptide (IFP) and two heptad-repeat domains preceding the transmembrane domain (TM) are distinguished in the structure of S2-subunit (Fig. 1). IFPs of the SARS-CoV and SARS-CoV-2 are identical [35].

SARS-CoV-2 S glycoprotein possesses also a furin-like cleavage site (PRRA) at the boundary between the S1/S2 subunits. Such a site is absent in SARS-CoV and MERS-CoV (Fig. 1) [12, 35, 53].

The surface of the spike protein of SARS-CoV-2, similarly to other coronavirus spike proteins, is dominated by host-derived glycans with each trimer displaying N-linked glycosylation sites. These oligosaccharides play a role in protein folding (influence on priming of S protein by host proteases) and immune evasion (modulation of recognition by antibodies).

SARS-CoV-2 S protein comprises 22 glycosylation sequons per protomer and 16 of them were resolved by Cryo-Electron Microscope map. 20 out of 22 SARS-CoV-2 N-linked glycosylation sequons are also conserved in SARS-CoV. 9 out of 13 glycans in the S1 subunit and all 9 glycans in the S2 subunit are conserved in both, SARS-CoV-2 S and SARS-CoV S proteins [12]. Furthermore, according to Walls *et al.* (2020), S2 N-linked glycosylation sequons are mostly conserved across SARSr-CoV S glycoproteins, suggesting that accessibility of the fusion machinery to antibodies will be comparable among these viruses [69]. However, Watanabe *et al.* (2020) demonstrated that SARS-CoV-2 S glycans differ from typical host glycan processing, which may have implications in viral pathobiology and vaccine design [72].

Other viral molecules anchored in the viral envelope are envelope (E) and membrane (M) proteins.

Data on envelope (E) protein of SARS-CoV and MERS-CoV indicate that E protein is a small 76–109 amino acids molecule, which contains hydrophilic amino terminus composed of 7–12 amino acids, hydrophobic transmembrane domain (TMD) of 25 amino acids, and hydrophilic carboxyl terminus. The hydrophobic region of the TMD contains at least one predicted amphipathic α-helix that oligomerizes to form an ion-conductive pore in viral membranes. The E protein can interact with other viral proteins, e.g. with viral M, N and S proteins [1, 43, 67, 60]. The E protein is engaged in several aspects of the virus replication cycle, such as assembly, budding, envelope formation, and pathogenesis. Absence, or inactivation, of E protein results in attenuation of viruses, due to alterations in either virion morphology or tropism. Besides its morphogenetic role, E protein can function as a viroporin with membrane-permeabilizing activity facilitating virus release from infected cells [68]. The data on envelope protein of SARS-CoV-2 are extremely limited. There are only *in silico* data concerning the interactome of the SARS-CoV-2 E protein. Nsp1, nsp8, ORF3a, ORF3a, ORF3b, ORF7b, ORF8a, ORF8b and ORF9b proteins probably interact with the SARS-CoV-2 E protein [18].

The membrane (M) protein of SARS-CoV and MERS-CoV is ~25–30 kDa with N-terminal glycosylated ectodomain and C-terminal endodomain. M is the most abundant structural protein present in the viral envelope. This dimeric protein can adopt two conformations. Elongated M protein is connected with rigidity, clusters of spikes and a relatively narrow range of membrane curvature, and compact M protein promotes flexibility and low spike density. Thus, M protein participates in defining the shape of the viral envelope. By its interaction with other viral proteins, M protein is engaged in replication and assembly of viral particles. By its cooperation with N protein, M protein stabilizes the nucleocapsid and the internal core of virions and participates in the viral assembly. The interaction of M protein with spike glycoprotein ensures the retention of S in the ER-Golgi intermediate compartment (ERGIC)/Golgi complex and its incorporation into new virions. Together with E protein, M protein composes the viral envelope and interaction between M and E proteins is sufficient for virus particle formation and release of virus-like particles (VLPs) [18, 43]. According to our knowledge as of May 2020, there is no specific data on the M protein of SARS-CoV-2.

The nucleocapsid protein (N) consists of three highly conserved parts: an N-terminal RNA-binding domain (NTD), a C-terminal dimerization domain (CTD), and an intrinsically disordered central Ser/Arg (SR)-rich linker for primary phosphorylation and potential interaction sites with other protein and protein-RNA partners. The primary functions of N protein are binding to the viral RNA genome and packing them into a long helical nucleocapsid structure. Besides forming nucleocapsid, N protein is also engaged in the regulation of viral RNA synthesis during replication/transcription and viral assembly and budding, resulting in complete virion formation. N protein also participates in host – pathogen interactions by impact on actin reorganization, host cell cycle progression, and apoptosis [76].

The overall structure of SARS-CoV-2 N protein is similar to other reported coronavirus nucleocapsid proteins. However, the surface charge distribution patterns of N-terminal region of SARS-CoV-2 N protein are different from the N protein of SARS-CoV. These differences dramatically change the surface characterizations of the protein, which may result in the RNA-binding cleft being adaptive to its RNA genome. Further comparison of the NTD of SARS-CoV-2 N protein with an equivalent domain of mild type virus HCoV-OC43 demonstrated a unique potential RNA binding pocket which can be a potential unique drug target [8, 31, 65].

Similar to SARS-CoV nucleoprotein, N protein of SARS-CoV-2 is a highly immunogenic viral antigen, capable of inducing protective immune responses against SARS-CoV-2 [31].

A set of non-structural and accessory coronavirus proteins (nsps) are participating in viral replication and interaction with host cells (listed in Table I).

One of the key non-structural protein is the RNA-dependent RNA polymerase (RdRp, also known as nsp12), which catalyses the synthesis of viral RNA, possibly with the assistance of nsp7 and nsp8 as cofactors. Nsp12 is composed of three subdomains: a fingers subdomain (residues L366 – to A581 and K621 to G679), a palm subdomain (residues T582 to P620 and T680 to Q815), and a thumb subdomain (residues H816 to E920) [20].

Table I
Functions of *Betacoronavirus* non-structural proteins

Protein	Function
nsp1	Inhibits host translation and gene expression by mRNA degradation and binding 40S ribosome subunit resulting in blocking innate immune response [29, 30, 64].
nsp2	Binds to prohibitin proteins, function not determined [11, 22]
nsp3	PL2pro multi-domain transmembrane protein, possessing:
	• ADRP activity, promotes cytokine expression;
	• PLPro/Deubiquitinase domain, cleaves viral polyprotein;
	• antagonist of IRF3 and NF-κB Signaling resulting in blocking host immune response;
	• Ubl1 and Ac domains, interact with N protein;
	• Ubl2, NAB, G2M, SUD, Y domains, unknown functions [9, 16, 19, 44, 54].
nsp4	Interacts with nsp3; induce the Formation of Double-Membrane Vesicles [2, 48].
nsp5	MoPro or 3CLpro processing of viral polyproteins [66].
nsp6	Potential transmembrane scaffold protein [40].
nsp7	Forms hexadecameric nsp7-nsp8 complex, essential co-factor of nsp12, may act as processivity clamp for RNA polymerase [34, 77].
nsp8	Forms hexadecameric nsp7-nsp8 complex, essential co-factor of nsp12, may act as primase [34, 77].
nsp9	Single-stranded RNA binding protein [17].
nsp10	Cofactor for nsp16 and nsp14, forms heterodimer with both and stimulates ExoN and S-adenosylmethionine-dependent (nucleoside-2'-O)-methyltransferase [6, 10, 13, 41].
nsp12	RNA-dependent RNA polymerase (RdRp) [34].
nsp13	Superfamily 1-like helicase (HEL1), RNA helicase, 5' triphosphatase [28].
nsp14	• C-terminal domain functions as a (guanine-N7) methyl transferase (N7-MTase) for mRNA capping.
	• N-terminal exoribonuclease (ExoN) domain displays a 3'–5' exoribonuclease proofreading activity [6, 41].
nsp15	NendoU, uridylate-specific endoribonuclease [78].
nsp16	S-adenosylmethionine-dependent (nucleoside-2'-O)-methyltransferase modifying the RNA cap at ribose 2'-O positions [6, 10].

The SARS-CoV-2 RdRp shares high homology with RdRp of SARS-CoV. Despite that, the overall architecture of nsp12-nsp7-nsp8 complex of SARS-CoV-2 is similar to that of SARS-CoV, few features distinguish them. In addition to the conserved architecture of the polymerase core of the viral polymerase family, nsp12 of SARS-CoV-2 possesses a newly identified β-hairpin domain at its N terminus [20].

5. *Betacoronavirus* Replication Cycle

Coronaviruses initiate infection by attaching to their specific receptors present on the surface of a susceptible host cell. The interaction between viral S protein and its cognate cellular receptor controls viral tissue tropism. On the mature virus, the S protein is present as a trimer, with three receptor-binding S1 heads sitting on the top of a trimeric S2 stalk (Fig. 1). To enter host cells, coronaviruses bind first to the cell surface and the attachment is mediated by interactions between the surface unit, S1 and its cellular receptor. As mentioned above, the ACE2 is the cognate cellular receptor for spike protein of SARS-CoV, while MERS-CoV binds to the cell surface by dipeptidyl peptidase 4 (DPP4) [52].

ACE2 has recently been identified as SARS-CoV-2 receptor [12, 39]. In turn, according to Ou X. *et al.* (2020), lentiviral pseudovirions expressing SARS-CoV-2 spike protein were able to transduce human cells expressing ACE2, but not those expressing the DPP4 or aminopeptidase N, indicating that the presence of DPP4 or aminopeptidase is not sufficient for SARS-CoV-2 attachment [47, 71].

Integrins may act as an alternative receptor for SARS-CoV-2, as on the surface of the spike protein of SARS-CoV-2 was discovered the motif Arg-Gly-Asp known to bind integrins. Such motif is absent in S glycoproteins of other coronaviruses [57].

Attachment of the viral S protein to host receptors (*via* S1) mediates endocytosis of the virus into the host cell [4]. Ou X. *et al.* (2020) has demonstrated that lentiviral pseudovirions expressing SARS-CoV-2 spike protein enter into host cells mainly through endocytosis, strongly suggesting that SARS-CoV-2 also penetrate the host cells by this mechanism [47].

In the next step of coronavirus replication, the fusion of the virus phospholipid envelope with the endosomal membrane, resulting in the release of nucleocapsid, into the host cell cytosol, where further stages of the replication cycle take place.

To induce the fusion of the membranes, SARS-CoV spike needs to be proteolytically activated. The SARS-CoV entry-activating proteases include cell surface protease TMPRSS2 and lysosomal proteases cathepsins [5, 58].

S protein cleavage occurs at two sites within the S2 subunit, with the first cleavage important for dissociation the S1 domain and the second for exposing the fusion peptide (cleavage at S2'). Cleavage at S2' exposes the fusion peptide that inserts into the membrane, which is followed by joining of two heptad repeats in S2 and forming of an antiparallel six-helix bundle [51]. The formation of this bundle allows for the mixing of viral and cellular membranes, resulting in fusion and ultimately release of the viral nucleocapsid into the cytoplasm. There the uncoating of viral genomic RNA takes place.

Similarly to SARS-CoV, SARS-CoV-2 entry to host cells also requires acid-dependent S protein priming by cellular proteases TMPRSS2, which entails S protein cleavage at the S1/S2 and the S2' site [39]. Further, as for SARS-CoV and MERS-CoV, cathepsin L also appears essential for priming of SARS-CoV-2 S protein in lysosome for releasing of the nucleocapsid into the cytoplasm [24, 71].

Unlike to SARS-CoV, the entry of SARS-CoV-2 into host cells is preactivated by the proprotein convertase furin, reducing virus dependence on cell proteases for entry. The inhibition of furin-dependent priming of S protein significantly decreases cell entry efficiency of SARS-CoV-2. The preactivation may have implications in enhanced SARS-CoV-2 entry in some type of cells (Fig. 1) [55].

The next step in the coronavirus replication cycle is the translation of polyproteins from ORF1a and ORF1b of the viral genomic RNA. There is a – 1 frameshift between ORF1a and ORF1b, leading to the production of two polypeptides (pp1a and pp1ab). Then polyproteins are proteolytically cleaved by virus-encoded proteases into 16 non-structural proteins (nsp1-16) that form the replicase / transcriptase complex (RTC), that directs and coordinates the replication and transcription of the viral genome. The replicative complex consists in RNA-dependent RNA polymerase (nsp12), the helicase/triphosphatase (nsp13), two unusual ribonucleases (nsp14, nsp15) and RNA-cap methyltransferases (nsp14, nsp16), which co-operate with other viral co-factors (nsp7, nsp8, and nsp10) to regulate their activity [63].

RTC is anchored in double-membrane vesicles (DMVs), which are coronavirus replicate structures. These 200–300 nm membraneous structures derive from the endoplasmic reticulum or Golgi apparatus [61].

The viral genome is a template not only for translation of pp1a and pp1ab polyproteins but also for replication, which is mediated by nsp12 and leads to the synthesis of negative-sense RNA intermediates. Negative-sense RNA intermediates serve as templates for the synthesis of positive-sense genomic RNA (gRNA) and subgenomic RNAs (sgRNAs). A subset of 9 subgenomic RNAs, including those encoding all structural proteins, is produced through discontinuous transcription. These subgenomic (–)RNAs are then transcribed into subgenomic (+)mRNAs, from which, structural proteins are translated [33]. Each viral transcript has a 5'-cap structure and a 3' poly(A) tail. In addition to the canonical genomic and 9 subgenomic RNAs, SARS-CoV-2 produces transcripts encoding unknown ORFs with fusion, deletion, and/or frameshift. S, E and M proteins, inserted into the endoplasmic reticulum, move along the secretory pathway into the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). There, viral genomes encapsidated by N protein bud into membranes of the ERGIC containing viral structural proteins, forming mature virions. Following assembly, virions are transported to the cell surface in vesicles and released by exocytosis.

6. Pathogenesis of SARS-CoV-2

6.1. Tissue and cellular pathogenesis

As mentioned above, SARS-CoV-2 binds to ACE2, the primary physiological function of which is the maturation of angiotensin, a peptide hormone, that controls vasoconstriction and blood pressure [37]. The ACE2 is broadly expressed in cells of the nasal mucosa, bronchus, lung, heart, esophagus, kidney, stomach, bladder, and ileum, and therefore these human organs are all vulnerable to SARS-CoV-2 [36].

SARS-CoV-2 is transmitted predominantly via respiratory droplet, by contact, and potentially by the faecal-oral route [49]. Primary viral replication is presumed to occur in the mucosal epithelium of the upper respiratory tract, with further multiplication in the lower respiratory tract. Most infections are controlled at this point, and seems asymptomatic at least concerning the respiratory tract. Thus, patients may be asymptomatic or experience mild, moderate or severe symptoms, associated with or without pneumonia [15, 23, 74]. Major pathological findings in the respiratory tract of SARS-CoV-2-infected patients include diffuse alveolar damage with varying degrees of acute exudative features including edema and hyaline membranes and fibrosis. Interstitial mononuclear inflammatory infil-

trates, dominated by lymphocytes, could be observed in the lungs, as well. In turn, multinucleated syncytial cells with atypical enlarged pneumocytes characterized by large nuclei, amphophilic granular cytoplasm, and prominent nucleoli identified in the intra-alveolar spaces indicate viral cytopathic-like changes. Some patients have also exhibited non-respiratory symptoms such as acute liver and heart injury, kidney failure, diarrhea, implying multiple organ involvement. The pathological features of COVID-19 resemble those seen in SARS-CoV or MERS-CoV infection [27, 74].

6.2. Molecular basis of pathogenesis

Several viral proteins affect the homeostasis of an infected human host at the molecular level. It was demonstrated that SARS-CoV nsp1 protein mediates host mRNA decay resulting in the inhibition of host translation [26]. SARS-CoV nsp15 protein interacts with host tumour suppressor retinoblastoma (pRb) protein, resulting in an increased proportion of cells in the S phase of the cycle [3]. SARS-CoV nsp3a protein may induce endoplasmic reticulum stress and degradation of the type 1 interferon receptor and thus inhibit type I IFN signalling and antiviral defences [42]. SARS-CoV papain-like protease mediates inhibition of host ISG15 and IRF3 [14, 38]. SARS-CoV M protein mediates the inhibition of host TRAF3 [59]. Therefore, SARS-CoV influence host gene expression, cell-cycle regulation and innate immune response by blocking IFN pathway.

Concerning SARS-CoV-2, an affinity-purification mass spectrometry (AP-MS) analysis identified 332 high-confidence protein interactions between 26 SARS-CoV-2 proteins and human proteins [21]. Identified interactions include several complexes and biological processes, such as DNA replication, epigenetic and gene expression regulators, vesicle trafficking, lipid modification, RNA processing and regulation, ubiquitin ligases, signalling, nuclear transport machinery, cytoskeleton, mitochondria, and extracellular matrix [21]. SARS-CoV-2 proteins interact also with innate immune pathways; *e.g.* with IFN pathway, with NF-κB pathway, with E3 ubiquitin ligases (that regulate antiviral innate immune signalling) or with the interferon-inducible mRNA nuclear export complex. SARS-CoV-2 also affects host translation machinery: N protein binds G3BP1/2 (the stress granule proteins), and to other host mRNA binding proteins, including the mTOR-regulated translational repressor LARP1, the casein kinase CK2 (involved in mRNA stability), and mRNA decay factors UPF1 and MOV10. Moreover, SARS-CoV-2 E protein binds to two bromodomain proteins (BRD2 and BRD that bind acetylated histones and regulate gene transcription [21].

6.3. Immunopathological changes in COVID-19

The patients with COVID-19 have a disturbance of the immune system. Immunologic changes include alteration in the number and functioning of immune system cells: the infection can cause cytotoxic T cell lymphopenia, eosinopenia, the increase of acute phase reactants, or disturbed T cell response.

A large number of data suggests that pathogenesis of SARS-CoV-2 can also be related to cytokine storm syndrome, which is a systemic inflammatory response to antigens of the pathogen. COVID-19 infection showed increased levels of plasma pro-inflammatory mediators, including IL1-β, IL1RA, IL7, IL8, IL9, IL10, basic FGF2, GCSF, GMCSF, IFNγ, IP10, MCP1, MIP1α, MIP1β, PDGFB, TNFα, and VEGFA [46]. The cytokine storm is a major factor for disseminated intravascular coagulation and acute respiratory distress syndrome (ARDS). The latter, due to cytokine storm, triggers a damaging attack to the body causing failure of multiple organs subsequently and leads to death.

7. Conclusion

SARS-CoV-2 is an emerging human coronavirus causing a pandemic in 2020. So far, there is no effective antiviral drug against SARS-CoV-2. In many aspects, it appears to similar to other coronaviruses: SARS-CoV and MERS-CoV. Nevertheless, some differences were observed concerning genome organization, protein structure and pathogenesis. Unlike spike protein of SARS-CoV, SARS-CoV-2 S glycoprotein is preactivated by proprotein convertase furin, reducing its dependence on target cell proteases for entry. Subtle differences in the structure of SARS-CoV-2 RNA-dependent RNA polymerase, RDB of spike protein or nucleocapsid protein are also observed. The significance of such differences concerning the pathogenesis of SARS-CoV-2 needs to be investigated. These features may contribute to the wide spread of SARS-CoV-2 but may also represent the putative targets for the design of specific therapy against SARS-CoV-2.

The mechanisms responsible for pathological lesions of organs non-connected with respiratory tracts should be also investigated.

References

1. Alvarez E., DeDiego M.L., Nieto-Torres J.L., Jiménez-Guardeño J.M., Marcos-Villar L., Enjuanes L.: The envelope protein of severe acute respiratory syndrome coronavirus interacts with the non-structural protein 3 and is ubiquitinated. *Virology*, **402**, 281–291 (2010)

2. Angelini M.M., Akhlaghpour M., Neuman B.W., Buchmeier M.J.: Severe acute respiratory syndrome coronavirus nonstructural proteins 3, 4, and 6 induce double-membrane vesicles. *mBio*, **4**, e00524–00513 (2013)
3. Bhardwaj K., Liu, P., Leibowitz J.L., Kao C.C.: The coronavirus endoribonuclease Nsp15 interacts with retinoblastoma tumor suppressor protein. *J. Virol*. **86**, 4294–4304 (2012)
4. Bosch B.J., Bartelink W., Rottier P.J.: Cathepsin L functionally cleaves the severe acute respiratory syndrome coronavirus class I fusion protein upstream of rather than adjacent to the fusion peptide. *J. Virol*. **82**, 8887–8890 (2008)
5. Bosch B.J., van der Zee R., de Haan C.A., Rottier P.J.: The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex. *J. Virol*. **77**, 8801–8811 (2003)
6. Bouvet M., Debarnot C., Imbert I., Selisko B., Snijder E.J., Canard B., Decroly E.: In vitro reconstitution of SARS-coronavirus mRNA cap methylation. *PLoS Pathog*. **6**, e1000863 (2010)
7. Chan J.F.-W., Kok K.-H., Zhu Z., Chu H., To K.K.-W., Yuan S., Yuen K.-Y.: Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerg. Microbes Infect*. **9**, 221–236 (2020)
8. Chang C.-k., Huang T.-h. i wsp.: Modular organization of SARS coronavirus nucleocapsid protein. *J. Biomed. Sci.* **13**, 59–72 (2006)
9. Chatterjee A., Johnson M.A., Serrano P., Pedrini B., Joseph J.S., Neuman B.W., Saikatendu K., Buchmeier M.J., Kuhn P., Wüthrich K.: Nuclear magnetic resonance structure shows that the severe acute respiratory syndrome coronavirus-unique domain contains a macrodomain fold. *J. Virol.* **83**, 1823–1836 (2009)
10. Chen Y., Guo D. i wsp.: Biochemical and structural insights into the mechanisms of SARS coronavirus RNA ribose 2'-O-methylation by nsp16/nsp10 protein complex. *PLoS Pathog*. **7**, e1002294 (2011)
11. Cornillez-Ty C.T., Liao L., Yates J.R., 3rd, Kuhn P., Buchmeier M.J.: Severe acute respiratory syndrome coronavirus nonstructural protein 2 interacts with a host protein complex involved in mitochondrial biogenesis and intracellular signaling. *J. Virol.* **83**, 10314–10318 (2009)
12. Coutard B., Valle C., de Lamballerie X., Canard B., Seidah N.G., Decroly E.: The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. *Antiviral Res*. **176**, 104742 (2020)
13. Decroly E., Canard B. i wsp.: Crystal structure and functional analysis of the SARS-coronavirus RNA cap 2'-O-methyltransferase nsp10/nsp16 complex. *PLoS Pathog*. **7**, e1002059 (2011)
14. Devaraj S.G., Wang N., Chen Z., Tseng M., Barretto N., Lin R., Peters C.J., Tseng C.T., Baker S.C., Li K.: Regulation of IRF-3-dependent innate immunity by the papain-like protease domain of the severe acute respiratory syndrome coronavirus. *J. Biol. Chem*. **282**, 32208–32221 (2007)
15. Dong X., Cao Y.Y., Lu X.X., Zhang J.J., Du H., Yan Y.Q., Akdis C.A., Gao Y.D.: Eleven faces of coronavirus disease 2019. *Allergy*, **75**, 1699–1709 (2020)
16. Egloff M.-P., Canard B. i wsp.: Structural and functional basis for ADP-ribose and poly(ADP-ribose) binding by viral macro domains. *J. Virol.* **80**, 8493–8502 (2006)
17. Egloff M.P., Ferron F., Campanacci V., Longhi S., Rancurel C., Dutartre H., Snijder E.J., Gorbalenya A.E., Cambillau C., Canard B.: The severe acute respiratory syndrome-coronavirus replicative protein nsp9 is a single-stranded RNA-binding subunit unique in the RNA virus world. *Proc. Natl. Acad. Sci. USA*, **101**, 3792–3796 (2004)
18. Escors D., Ortego J., Laude H., Enjuanes L.: The Membrane M Protein Carboxy Terminus Binds to Transmissible Gastroenteritis Coronavirus Core and Contributes to Core Stability. *J. Virol.* **75**, 1312–24 (2001)
19. Frieman M., Ratia K., Johnston R.E., Mesecar A.D., Baric R.S.: Severe acute respiratory syndrome coronavirus papain-like protease ubiquitin-like domain and catalytic domain regulate antagonism of IRF3 and NF-kappaB signaling. *J. Virol.* **83**, 6689–6705 (2009)
20. Gao Y., Rao, Z. i wsp.: Structure of the RNA-dependent RNA polymerase from COVID-19 virus. *Science*, **368**, 779–782 (2020)
21. Gordon D.E., Krogan N.J. i wsp.: A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature*, **583**, 459–468 (2020)
22. Graham R.L., Sims A.C., Brockway S.M., Baric R.S., Denison M.R.: The nsp2 replicase proteins of murine hepatitis virus and severe acute respiratory syndrome coronavirus are dispensable for viral replication. *J. Virol.* **79**, 13399–13411 (2005)
23. Gu J., Korteweg C.: Pathology and pathogenesis of severe acute respiratory syndrome. *Am. J. Pathol.* **170**, 1136–1147 (2007)
24. Hoffmann M., Kleine-Weber H., Pöhlmann S.: A multibasic cleavage site in the spike protein of SARS-CoV-2 Is essential for infection of human lung cells. *Mol. Cell*. **78**, 779–784.e775 (2020)
25. Hoffmann M., Pöhlmann S. i wsp.: SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*, **181**, 271–280.e278 (2020)
26. Huang C., Lokugamage K.G., Rozovics J.M., Narayanan K., Semler B.L., Makino S.: SARS coronavirus nsp1 protein induces template-dependent endonucleolytic cleavage of mRNAs: viral mRNAs are resistant to nsp1-induced RNA cleavage. *PLoS Pathog*. **7**, e1002433 (2011)
27. Huang C., Cao B. i wsp.: Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*, **395**, 497–506 (2020)
28. Ivanov K.A., Thiel V., Dobbe J.C., van der Meer, Y., Snijder E.J., Ziebuhr J.: Multiple enzymatic activities associated with severe acute respiratory syndrome coronavirus helicase. *J. Virol*. **78**, 5619–5632 (2004)
29. Kamitani W., Huang C., Narayanan K., Lokugamage K.G., Makino S.: A two-pronged strategy to suppress host protein synthesis by SARS coronavirus Nsp1 protein. *Nat. Struct. Mol. Biol*. **16**, 1134–1140 (2009)
30. Kamitani W., Narayanan K., Huang C., Lokugamage K., Ikegami T., Ito N., Kubo H., Makino S.: Severe acute respiratory syndrome coronavirus nsp1 protein suppresses host gene expression by promoting host mRNA degradation. *Proc. Natl. Acad. Sci. USA*, **103**, 12885–12890 (2006)
31. Kang S., Chen S. i in.: Crystal structure of SARS-CoV-2 nucleocapsid protein RNA binding domain reveals potential unique drug targeting sites. *Acta Pharm. Sin B*. **10**, 1228–1238 (2020)
32. Khailany R.A., Safdar M., Ozaslan M.: Genomic characterization of a novel SARS-CoV-2. *Gene Rep*. 100682 (2020)
33. Kim D., Lee J.Y., Yang J.S., Kim J.W., Kim V.N., Chang H.: The Architecture of SARS-CoV-2 Transcriptome. *Cell*, **181**, 914–921. e910 (2020)
34. Kirchdoerfer R.N., Ward A.B.: Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. *Nat. Commun*. **10**, 2342 (2019)
35. Lan J., Wang X. i wsp.: Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature*, **581**, 215–220 (2020)
36. Li M.-Y., Li L., Zhang Y., Wang X.-S.: Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues. *Infect. Dis. Poverty*. **9**, 45 (2020)

37 Li Y., Zhou W., Yang L., You R.: Physiological and pathological regulation of ACE2, the SARS-CoV-2 receptor. *Pharmacol Res*. **157**, 104833–104833 (2020)
38. Lindner H.A., Lytvyn V., Qi H., Lachance P., Ziomek E., Ménard R.: Selectivity in ISG15 and ubiquitin recognition by the SARS coronavirus papain-like protease. *Arch. Biochem. Biophys*. **466**, 8–14 (2007)
39. Luan J., Lu Y., Jin X., Zhang L.: Spike protein recognition of mammalian ACE2 predicts the host range and an optimized ACE2 for SARS-CoV-2 infection. *Biochem Biophys. Res. Commun*. **526**, 165–169 (2020)
40. Lundin A., Trybala E. i wsp.: Targeting membrane-bound viral RNA synthesis reveals potent inhibition of diverse coronaviruses including the middle East respiratory syndrome virus. *PLoS Pathog*. **10**, e1004166–e1004166 (2014)
41. Ma Y., Wu L., Shaw N., Gao Y., Wang J., Sun Y., Lou Z., Yan L., Zhang R., Rao Z.: Structural basis and functional analysis of the SARS coronavirus nsp14-nsp10 complex. *Proc. Natl. Acad. Sci. USA*, **112**, 9436–9441 (2015)
42. Minakshi R., Padhan K., Rani M., Khan N., Ahmad F., Jameel S.: The SARS Coronavirus 3a protein causes endoplasmic reticulum stress and induces ligand-independent downregulation of the type 1 interferon receptor. *PLoS One*, **4**, e8342 (2009)
43. Mortola E., Roy P.: Efficient assembly and release of SARS coronavirus-like particles by a heterologous expression system. *FEBS Lett*. **576**, 174–178 (2004)
44. Neuman B.W., Kuhn P. i wsp.: Proteomics analysis unravels the functional repertoire of coronavirus nonstructural protein 3. *J. Virol*. **82**, 5279–5294 (2008)
45. Neuman B.W., Buchmeier M.J. i wsp.: A structural analysis of M protein in coronavirus assembly and morphology. *J. Struct. Biol*. **174**, 11–22 (2011)
46. Nile S.H., Nile A., Qiu J., Li L., Jia X., Kai G.: COVID-19: Pathogenesis, cytokine storm and therapeutic potential of interferons. *Cytokine Growth Factor Rev*. **53**, 66–70 (2020)
47. Ou X., Qian Z. i wsp.: Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat. Commun*. **11**, 1620 (2020)
48. Oudshoorn D., Rijs K., Limpens R., Groen K., Koster A.J., Snijder E.J., Kikkert M., Bárcena M.: Expression and Cleavage of Middle East Respiratory Syndrome Coronavirus nsp3–4 Polyprotein Induce the Formation of Double-Membrane Vesicles That Mimic Those Associated with Coronaviral RNA Replication. *mBio*, **8** (2017)
49. Park S.E.: Epidemiology, virology, and clinical features of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2; Coronavirus Disease-19). *Clin Exp Pediatr*. **63**, 119–124 (2020)
50. Phan T.: Genetic diversity and evolution of SARS-CoV-2. *Infect Genet Evol*. **81**, 104260 (2020)
51. Qian Z., Dominguez S.R., Holmes K.V.: Role of the spike glycoprotein of human Middle East respiratory syndrome coronavirus (MERS-CoV) in virus entry and syncytia formation. *PLoS One*, **8**, e76469 (2013)
52. Raj V.S., Haagmans B.L. i wsp.: Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature*, **495**, 251–254 (2013)
53. Schoeman D., Fielding B.C.: Coronavirus envelope protein: current knowledge. *Virol. J*. **16**, 69 (2019)
54. Serrano P., Johnson M.A., Chatterjee A., Neuman B.W., Joseph J.S., Buchmeier M.J., Kuhn P., Wüthrich K.: Nuclear magnetic resonance structure of the nucleic acid-binding domain of severe acute respiratory syndrome coronavirus nonstructural protein 3. *J. Virol*. **83**, 12998–13008 (2009)
55. Shang J., Wan Y., Luo C., Ye G., Geng Q., Auerbach A., Li F.: Cell entry mechanisms of SARS-CoV-2. *Proc. Natl. Acad. Sci. USA*, **117**, 11727–11734 (2020)
56. Shang J., Ye G., Shi K., Wan Y., Luo C., Aihara H., Geng Q., Auerbach A., Li F.: Structural basis of receptor recognition by SARS-CoV-2. *Nature*, **581**, 221–224 (2020)
57. Sigrist C.J., Bridge A., Le Mercier P.: A potential role for integrins in host cell entry by SARS-CoV-2. *Antiviral Res*. **177**, 104759 (2020)
58. Simmons G., Gosalia D.N., Rennekamp A.J., Reeves J.D., Diamond S.L., Bates P.: Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. *Proc. Natl. Acad. Sci. USA*, **102**, 11876–11881 (2005)
59. Siu K.L., Kok K.H., Ng M.H., Poon V.K., Yuen K.Y., Zheng B.J., Jin D.Y.: Severe acute respiratory syndrome coronavirus M protein inhibits type I interferon production by impeding the formation of TRAF3.TANK.TBK1/IKKepsilon complex. *J. Biol. Chem*. **284**, 16202–16209 (2009)
60. Siu Y.L., Nal B. i wsp.: The M, E, and N structural proteins of the severe acute respiratory syndrome coronavirus are required for efficient assembly, trafficking, and release of virus-like particles. *J. Virol*. **82**, 11318–11330 (2008)
61. Snijder E.J., van der Meer, Y., Zevenhoven-Dobbe J., Onderwater J.J.M., van der Meulen J., Koerten H.K., Mommaas A.M.: Ultrastructure and Origin of Membrane Vesicles Associated with the Severe Acute Respiratory Syndrome Coronavirus Replication Complex. *J. Virol*. **80**, 5927–40 (2006)
62. Srinivasan S., Cui H., Gao Z., Liu M., Lu S., Mkandawire W., Narykov O., Sun M., Korkin D.: Structural Genomics of SARS-CoV-2 Indicates Evolutionary Conserved Functional Regions of Viral Proteins. *Viruses*, **12**, 360 (2020)
63. Subissi L., Imbert I., Ferron F., Collet A., Coutard B., Decroly E., Canard B.: SARS-CoV ORF1b-encoded nonstructural proteins 12–16: replicative enzymes as antiviral targets. *Antiviral Res*. **101**, 122–130 (2014)
64. Tanaka T., Kamitani W., DeDiego M.L., Enjuanes L., Matsuura Y.: Severe acute respiratory syndrome coronavirus nsp1 facilitates efficient propagation in cells through a specific translational shutoff of host mRNA. *J. Virol*. **86**, 11128–11137 (2012)
65. Tang T.-K., Wu M.P.J., Chen S.-T., Hou M.-H., Hong M.-H., Pan F.-M., Yu H.-M., Chen J.-H., Yao C.-W., Wang A.H.J.: Biochemical and immunological studies of nucleocapsid proteins of severe acute respiratory syndrome and 229E human coronaviruses. *Proteomics*, **5**, 925–937 (2005)
66. Tomar S., Johnston M.L., St John S.E., Osswald H.L., Nyalapatla P.R., Paul L.N., Ghosh A.K., Denison M.R., Mesecar A.D.: Ligand-induced Dimerization of Middle East Respiratory Syndrome (MERS) Coronavirus nsp5 Protease (3CLpro): IMPLICATIONS FOR nsp5 REGULATION AND THE DEVELOPMENT OF ANTIVIRALS. *J. Biol. Chem*. **290**, 19403–19422 (2015)
67. Tseng Y.T., Wang S.M., Huang K.J., Wang C.T.: SARS-CoV envelope protein palmitoylation or nucleocapid association is not required for promoting virus-like particle production. *J. Biomed. Sci*. **21**, 4 (2014)
68. Vennema H., Godeke G.J., Rossen J.W., Voorhout W.F., Horzinek M.C., Opstelten D.J., Rottier P.J.: Nucleocapsid-independent assembly of coronavirus-like particles by co-expression of viral envelope protein genes. *EMBO J*. **15**, 2020–2028 (1996)
69. Walls A.C., Park Y.J., Tortorici M.A., Wall A., McGuire A.T., Veesler D.: Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell*, **181**, 281–292.e286 (2020)
70. Wang C., Liu Z., Chen Z., Huang X., Xu M., He T., Zhang Z.: The establishment of reference sequence for SARS-CoV-2 and variation analysis. *J. Med. Virol*. **92**, 667–674 (2020)

71. Wang H., Yang P., Liu K., Guo F., Zhang Y., Zhang G., Jiang C.: SARS coronavirus entry into host cells through a novel clathrin- and caveolae-independent endocytic pathway. *Cell Res*. **18**, 290–301 (2008)
72. Watanabe Y., Allen J.D., Wrapp D., McLellan J.S., Crispin M.: Site-specific glycan analysis of the SARS-CoV-2 spike. *Science*, eabb9983 (2020)
73. Wrapp D., Wang N., Corbett K.S., Goldsmith J.A., Hsieh C.L., Abiona O., Graham B.S., McLellan J.S.: Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science*, **367**, 1260–1263 (2020)
74. Xu Z., Wang F.S.: Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir. Med*. **8**, 420–422 (2020)
75. Yan R., Zhang Y., Li Y., Xia L., Guo Y., Zhou Q.: Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science*, **367**, 1444–1448 (2020)
76. Zeng W., Jin T. i wsp.: Biochemical characterization of SARS-CoV-2 nucleocapsid protein. *Biochem Biophys. Res. Commun*. **527**, 618–623 (2020)
77. Zhai Y., Sun F., Li X., Pang H., Xu X., Bartlam M., Rao Z.: Insights into SARS-CoV transcription and replication from the structure of the nsp7-nsp8 hexadecamer. *Nat. Struct. Mol. Biol*. **12**, 980–986 (2005)
78. Zhang L., Li L., Yan,L., Ming Z., Jia Z., Lou Z., Rao Z.: Structural and Biochemical Characterization of Endoribonuclease Nsp15 Encoded by Middle East Respiratory Syndrome Coronavirus. *J. Virol*. **92**, e00893–18 (2018)