



An Introduction to SARS Coronavirus 2; Comparative Analysis with MERS and SARS Coronaviruses: A Brief Review

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Abstract

Since the 1970 the replication and pathogenesis mechanism of different coronaviruses have been studied. In 2002-2003, SARS (Severe Acute Respiratory Syndrome coronavirus) in China emerged which resulted in 8098 cases and 774 deaths. About 10 years later in 2012, the MERS (Middle East Respiratory Syndrome coronavirus) spread in Middle Eastern countries and leads to infection in 2465 cases. In Dec 2019, another acute respiratory disease caused by a novel coronavirus named SARS-2 emerged in Wuhan, China. The virus is assumed to be mainly transmitted by respiratory droplets. Travels and communications leads to high prevalence of COVID-19 (Coronavirus Disease 2019) in the world, and currently in Iran. The current review was conducted to compare the virus structure, genome organization, virus life cycle, pathogenesis and prediction the future of COVID-19.

Keywords: Pandemic; COVID-19; Viral infection

Introduction

Coronaviruses are a large family and a subset of Nidoviral order, Coronaviridae family, Coronavirinae subfamily. These viruses can cause a disease ranging from the common cold to more severe respiratory diseases and respiratory distress or acute respiratory distress syndrome (ARDS). Coronaviruses are enveloped, positive single-stranded RNA virus and naturally infect mammals and birds (1, 2). The coronavirus in human was isolated from the respiratory secretions of a patient with a common cold for the first time in the 1960s by Tyrell and Bynoe (3). The Coronavirinae subfamily has four genera includes *Alphacoronavirus*, *Betacoronavirus*, *Gammacoro-*

navirus and *Deltacoronavirus* (4). *Alphacoronavirus* and *Betacoronavirus* mostly infect mammals and especially bat, while *Gammacoronavirus* and *Deltacoronavirus* can infects birds. Bats could be great host for different types of coronaviruses based on geographical region and bat type (5). These animals appear to be the natural reservoir for coronaviruses (6). Among seven coronaviruses that can infect human, the *Betacoronavirus* causes serious and deadly respiratory diseases, while the *Alphacoronavirus* diseases are mild (7).

In 2002, an acute respiratory illness caused by a coronavirus emerged in China which was named the severe acute respiratory syndrome (SARS) (8).

The SARS was the causative agent of the severe acute infection in respiratory tract. The SARS epidemic lead to the 8098 infections during 2002-2003 and 774 death (9). Animal coronaviruses are capable of generating mutant viruses which they can pass thought other hosts such as human (10). In 2012 another emergence coronavirus, the Middle East Respiratory Syndrome (MERS) appeared and spread to the Middle East. This virus was isolated from lung of patients with pneumonia and acute renal failure concurrently by an Egyptian virologist (Ali Mohammad Zaki) for the first time in Jordan and Saudi Arabia in Sep 2012 (11). MERS was transmitted to humans thought infected camels and has 35% mortality rate (12). It caused 2465 confirmed cases worldwide, and the virus is still circulating by lower severity (13). In Dec 2019, another severe acute respiratory disease spread in Wuhan, China caused by a new coronavirus named SARS-2. The disease spread rapidly in China and other countries. On 30 Jan 2020, WHO enounce an international emergency in relation to this disease, and on 12 Feb 2020, the new coronavirus disease was named Coronavirus disease 2019 or COVID-19 (14). The disease transmitted mainly by respiratory droplets

and cause a wide range of symptoms (15). Epidemiological research conducted in Wuhan has shown an early connection between the seafood market and most of the patients who worked or visited these markets. However, cases of human-to-human transmission have increased the prevalence of the disease (16). The current review was conducted to compare the virus structure, genome organization, virus life cycle, pathogenesis and prediction of the future of COVID-19.

Genome Order

Coronaviruses are classified into four genera, including *Alphacoronavirus*, *Betacoronavirus*, *Gamma-coronavirus* and *Deltacoronavirus* as mentioned earlier. NL63, 229E belong to the *Alphacoronaviruses*, and HKU1, OC43, SARS and MERS, SARS-2 belong to the *Betacoronaviruses*. The coronavirus genome contains about 26,000 to 32,000 base pair and 6 to 11 open reading frames (ORFs) (17). The first ORF encompasses approximately 67% of the entire genome and encodes 16 unstructured proteins (nsp), while the rest of the ORFs encode structural an accessory proteins (Fig. 1) (18).

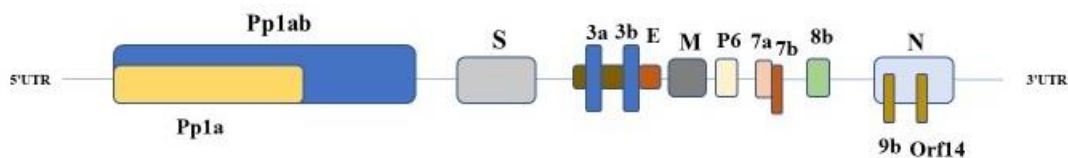


Fig. 1: The genome map of COVID-19. Schematic diagram of the genome structure and the encoded proteins pp1ab and pp1a for the IVDC-HB-01/2019 (HB01) strain. The largest gene, orf1ab, encodes a pp1ab protein containing 15 nsp. The pp1a protein encoded by the orf1a gene also contains 10 nsp. Structural proteins are encoded by four structural genes, and accessory genes are placed among structural genes

Studies on SARS-2 genome showed a quite similarity to SARS, although there were significant differences, such as presence of protein 8a, 8b in SARS but not in SARS-2 (19). Length of 8b and 3b proteins in SARS is 84 and 154 amino acids, but ORF 8 and 3b in SARS-2 has 121 and 22 amino acids, respectively (19, 20). In addition, phylogenetic studies on the whole genome of the *Betacoronavirus* revealed that SARS-2 had the highest similarity to SARS and was less similar to

MERS (21). Given the close relationship between SARS-2 and SARS, investigating the amino acid translocation in different proteins could justify the structural and functional differences between SARS-2 and SARS (20, 22).

Coronaviruses genomic RNA has methylated cap at the 5' end and a polyadenylated tail at 3' end, which allowing the viral RNA directly binds to the host ribosome for translation into amino acids. The coronavirus genome encodes RNA-

dependent RNA polymerase (RdRp) which is responsible for virus replication. The virus genome replicates and forms a long polyprotein in which contains all NSPs. This polyprotein divided into 16 distinct proteins by the virus protease activity (23).

Viral entry

Similar to the other coronaviruses, SARS-2 virion is spherical which contains structural proteins such as spike (S) on the surface, envelope (E), membrane (M) and nucleoprotein (N) (Fig. 2).

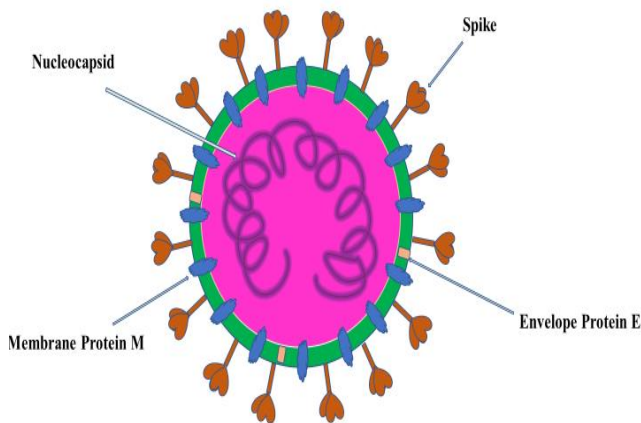


Fig. 2: Coronavirus structure (58)

The virus attached to the cell surface through S protein then underwent conformational alterations and fused with cell membranes (Fig. 3).

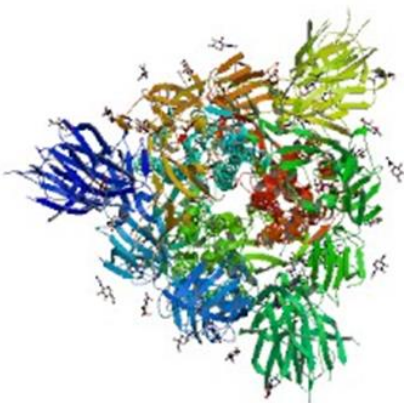


Fig. 3: Atomic structure of the Spike protein (DOI: 10.2210/pdb6VSB/pdb)

The Spike protein has two domains S1 and S2. The S1 is responsible for the attachment and the S2 for fusion. The SARS-2 S protein in epithelial cells under a proteolytic activity by host protease, serine 2 (TMPRSS2) cleaved into S1 and S2 subunits (24, 25). The SARS-2 attachment mediate by Angiotensin-converting enzyme 2 (ACE2), same as the SARS (20), while MERS binds to the Dipeptidyl peptidase-4 (DPP-4) (26). Although studies showed the SARS and SARS-2 Spike protein similarities, there are not found any cross-reactivity for neutralizing antibodies (27).

Spike protein is a type of transmembrane protein and its lengths ranged from 1,400-1,160 amino acids: the longest Spike protein were seen in Feline coronavirus (FCoV) and the shortest one in avian infectious bronchitis virus (IBV). In addition, this protein is highly glycosylated and contains 21-35 sites for N-glycosylation. All Spike proteins have two terminal domains: One N-terminal domain known as S1 and responsible for binding to the receptor, and one C-terminal domain or S2, responsible for virus or cell membrane fusion. The S2 domain is more conserve while the S1 subunit differs even among species of one type of coronavirus. The S1 subunit has two domains, an N-terminal domain (NTD) and a C-terminal domain (CTD) that both act as receptor-binding domain (RBD) (28). In addition, S proteins of coronaviruses have two heptad repeats (HR) in their S2 domain and these HRs are class I viral fusion proteins. This HR seems to conserve and useful in the fusion of the virus membrane with the host cell membrane (29, 30). Coronaviruses mostly use peptidases as cell receptors. The peptidases activity of the receptor dose does not seem to be necessity for the virus because virus entry occurs even in the absence of the enzymatic domain of these proteins. Many *Alphacoronaviruses* use aminopeptidases N (APN) as receptors and SARS and HCoV-NL63 and SARS-2 use angiotensin-converting enzyme 2 (ACE2) as receptor, which are found in the respiratory tract epithelium, lung parenchyma, vascular endothelium, kidney cells, and small intestine cells (31). On the other hand, MERS uses dipeptidyl peptidase 4, which is present in the

lower respiratory tract, kidneys, small intestine, liver and immune cells (32, 33).

The virus enters the host cell cytoplasm by using cathepsin protease activity, TMPRSS2 (a serine protease), or other proteases via proteolytic degradation mechanism and fusion of the viral membrane into the host cells (32). Fusion generally takes place within acidic endosomes. However, for some viruses, such as MHV, it can occur in the plasma membrane (28). The nature of the cellular proteases that cause cleavage of S protein is different between coronaviruses. In contrast, the S glycoprotein cleavage in the MERS mediated by Furin and uses two-step furin activation mechanism for membrane fusion (34).

Viral Immunity and Pathogenesis

Clinical manifestations in patients with COVID-19 include fever, cough, shortness of breath, fatigue, normal or decreased leukocyte counts, ground-glass opacity in radiographic examinations, which are similar to symptoms of SARS and MERS infections (35). Although the SARS-2 pathogenesis is not well understood so far, it may have a mechanism similar to that of SARS due to the higher sequence similarity (36).

Generally, viral antigens are processed by antigen-presenting cells (APCs), which have major histocompatibility complex (MHC), and then utilize by cytotoxic T lymphocytes (CTLs) system to defeat. Majority of SARS antigens presented by MHC-I and -II (37), while MERS just uses MHC-II (38). CD4⁺ and CD8⁺ levels in patients with SARS-2 infection were significantly reduced (39). Also, acute respiratory distress syndrome (ARDS) is one of the leading cause of death in COVID-19 (35). ARDS is a common immunological event for patients with SARS, SARS-2 and MERS infections (39). The major cause of ARDS is the cytokine storm that is an uncontrolled systemic inflammatory response induced by the release of high levels of cytokines (IFN- α , IFN- γ , IL-1 β , IL-6, IL-12, IL-18, IL-33, TNF- α , TGF β , and chemokines (CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10) (35, 40, 41). In patients with MERS and SARS, the serum levels of IL-6, IFN- α , and CCL5, CXCL8, CXCL-10 increased signifi-

cantly (42). ARDS and multiple organ failure eventually leads to death (39).

Coronaviruses and Central Nervous System

Early 2002 and 2003 studies revealed SARS infection in the brain (43, 44). Experimental studies using transgenic mice showed that SARS (45) and MERS (46) can enter the brain (possibly through the olfactory nerve) and inter-uterine injection of the virus can rapidly transfer it to the certain areas of the brain, including the thalamus and brain stem. The precise pathways that SARS and MERS enter the central nervous system are still unclear (46). Coronaviruses may first attack the peripheral nerve terminals and then reach the central nervous system via a synapse-related pathway (47, 48). Moreover, the tendency for the nervous system has been proven to be a common feature of coronaviruses and given the high similarity between SARS and SARS-2 (49). By considering the COVID-19 incubation period, its central nervous system involvement and destroying the medullary neurons is not far to understand, and possible reason for some symptoms such as headaches, nausea, and vomiting (49).

Therapeutic approaches and ACE2

Production of vaccine against Spike protein may be effective in the prevention of the disease. Binding of S protein to the ACE2 is the major mechanism in SARS-2 replication. The primary binding of S protein and TMPRSS2 serine protease enzyme is required for the entry and spread of SARS-2 through interaction with the ACE2 receptor (Fig. 4) (50). The use of inhibitory drugs TMPRSS2 can inhibit the entry of the virus (51, 52). This interaction site of the TMPRSS2 and S protein could be targeted and inhibited by small molecules or antibodies. SARS-CoV regulates ACE2 protein by binding to protein S and leads to severe pulmonary injury. Large amounts of ACE2 may competitively bind to SARS-CoV-2 leading to neutralization of the virus and even rescue cellular ACE2 activity that regulates the renin-angiotensin system through negative self-regulation and prevent lung damage. Increased ACE activity and reduced ACE2 availability in

lung injuries leads to the protection of the lung (53, 54). The use of the recombinant ACE2 could be suggested for the lung injuries preventing.

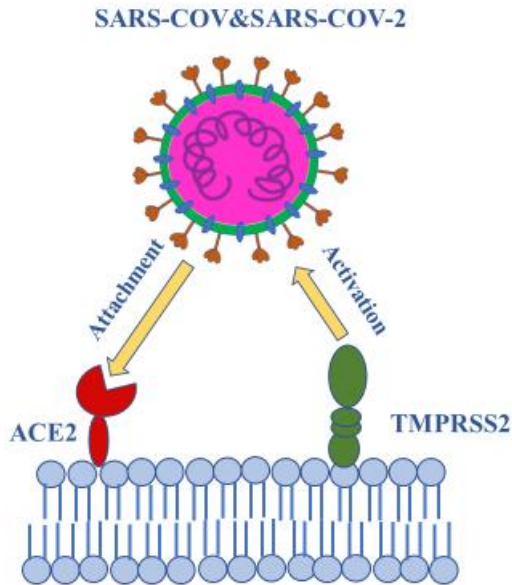


Fig. 4: ACE2 receptor and proteasome enzymes for fusion of virus membrane and cell

Future of COVID-19

Preventing the disease requires identifying carriers and isolating patients. COVID-19 could also be controlled similar to the SARS in 2002, but many asymptomatic patients can transfer infection easily. By Mar 15, 2020, WHO reports that there are 693224 infected with COVID-19 worldwide and 33106 deaths (55). Studies have shown the COVID-19 seasonal rotation in influenza (56). However, there is no data about the seasonal spreading of the COVID-19 or other epidemic coronaviruses. In this regard, the warmer months play an important role in reducing the spread of COVID-19. Therefore, the best way to deal with the disease is to prevent it from spreading (57).

Conclusion

The recent outbreak of COVID-19 such a major threat to people health and attracts scientists to defeat the virus. The outbreak has posed signifi-

cant challenges to public health, medical and social research. Many aspects of the SARS-CoV-2 such as virology, pathogenicity, and reservoir of virus are still not known clearly. Besides, providing appropriate medications and therapeutic targets to control the disease highly needed.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Mc Intosh Kenneth (2005). Coronaviruses in the Limelight. *Int J Infect Dis*, 191(4): 489–491.
2. Lau SK, Lee P, Tsang AK et al (2011). Molecular epidemiology of human coronavirus OC43 reveals evolution of different genotypes over time and recent emergence of a novel genotype due to natural recombination. *J Virol*, 85(21):11325-37.
3. Tyrrell D, Bynoe M, Hoorn B (1968). Cultivation of "difficult" viruses from patients with common colds. *Br Med J*, (5592): 606–610.
4. Gerna G, Campanini G, Rovida F et al (2006). Genetic variability of human coronavirus OC43-, 229E-, and NL63-like strains and their association with lower respiratory tract infections of hospitalized infants and

- immunocompromised patients. *J Med Virol*, 78(7):938-49.
5. Woo PC, Lau SK, Li KS et al (2006). Molecular diversity of coronaviruses in bats. *Virology*, 351(1):180-7.
 6. Peiris J, Lai S, Poon L et al (2003). Coronavirus as a possible cause of severe acute respiratory syndrome. *The Lancet*, 361(9366):1319-25.
 7. Spaan W, Cavanagh D, Horzinek M (1988). Coronaviruses: structure and genome expression. *J Gen Virol*, 69(12):2939-52.
 8. Balboni A, Battilani M, Prosperi S (2012). The SARS-like coronaviruses: the role of bats and evolutionary relationships with SARS coronavirus. *J Microbiol Sci*, 35(1):1-16.
 9. Peiris JSM, Chu C-M, Cheng VC-C, et al (2003). Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet*, 361(9371):1767-72.
 10. Khan G. (2013). A novel coronavirus capable of lethal human infections: an emerging picture. *Virol J*, 10(1):66.
 11. Zaki AM, Van Boheemen S, Bestebroer TM et al (2012). Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med*, 367(19):1814-20.
 12. Azhar EI, Hui DSC, Memish ZA et al (2019). The Middle East Respiratory Syndrome (MERS). *Infect Dis Clin North Am*, 33(4):891-905.
 13. Hui D, Madani T, Ntoumi F et al (2020). The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health-The latest 2019 novel coronavirus outbreak in Wuhan, China. *Int J Infect Dis*, 91:264-266.
 14. McIntosh, K., Hirsch, M.S. and Bloom, A. (2020). Coronavirus Disease 2019 (COVID-19), UpToDate. Available from: <https://www.uptodate.com/contents/coronavirus-disease-2019-covid-19-epidemiology-virology-clinical-features-diagnosis-and-prevention>
 15. World Health Organization (2020). WHO Disease outbreak news: Novel Coronavirus—Republic of Korea (ex-China). January 21, 2020. Available from: <https://www.who.int/csr/don/21-january-2020-novel-coronavirus-republic-of-korea-ex-china/en/>
 16. Liu, S.-L.; Saif, L. (2020). Emerging Viruses without Borders: The Wuhan Coronavirus. *Viruses*, 12, 130.
 17. Song Z, Xu Y, Bao L et al (2019). From SARS to MERS, thrusting coronaviruses into the spotlight. *Viruses*, 11(1):59.
 18. Cui J, Li F, Shi Z-L (2019). Origin and evolution of pathogenic coronaviruses. *Nat Rev Microbiol*, 17(3):181-92.
 19. Dhama K, Sharun K, Tiwari, et al (2020). Coronavirus Disease 2019 – COVID-19. Preprints (doi: 10.20944/preprints202003.0001.v2).
 20. Wrapp D, Wang N, Corbett KS et al (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science*, 13;367(6483):1260-3.
 21. Lu R, Zhao X, Li J et al (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The Lancet*, 395(10224):565-74.
 22. Zhu Z, Zhang Z, Chen W et al (2018). Predicting the receptor-binding domain usage of the coronavirus based on kmer frequency on spike protein. *Infect Genet Evol*, 61:183-4.
 23. Zhao L, Jha BK, Wu A et al (2012). Antagonism of the interferon-induced OAS-RNase L pathway by murine coronavirus ns2 protein is required for virus replication and liver pathology. *Cell Host Microbe*, 11(6):607-16.
 24. Hoffmann M, Kleine-Weber H, Schroeder S et al (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*, 181(2): 271-280.
 25. Glowacka I, Bertram S, Müller MA et al (2011). Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. *J Virol*, 85(9):4122-34.
 26. Zhou P, Yang X-L, Wang X-G et al (2020). Discovery of a novel coronavirus associated with the recent pneumonia outbreak in humans and its potential bat origin. *BioRxiv*, doi: 10.1038/s41586-020-2012-7.
 27. Yang Y, Peng F, Wang R et al (2020). The deadly coronaviruses: The 2003 SARS pandemic and the 2020 novel coronavirus epidemic in China. *J Autoimmun*, 109:102434.

28. Bosch BJ, van der Zee R, de Haan CA, Rottier PJ (2003). The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex. *J Virol*, 77(16):8801-11.
29. Xu Y, Cole DK, Lou Z et al (2004). Construct design, biophysical, and biochemical characterization of the fusion core from mouse hepatitis virus (a coronavirus) spike protein. *Protein Expr Purif*, 38(1):116-22.
30. Supekar VM, Bruckmann C, Ingallinella P et al (2004). Structure of a proteolytically resistant core from the severe acute respiratory syndrome coronavirus S2 fusion protein. *Proc Natl Acad Sci U S A*, 101(52):17958-63.
31. Donoghue M, Hsieh F, Baronas E et al (2000). A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ Res*, 87(5):E1-9.
32. Belouzard S, Chu VC, Whittaker GR (2009). Activation of the SARS coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. *Proc Natl Acad Sci U S A*, 106(14): 5871-5876.
33. Boonacker E, Van Noorden CJ (2003). The multifunctional or moonlighting protein CD26/DPPIV. *Eur J Cell Biol*, 82(2):53-73.
34. Millet JK, Whittaker GR. (2014). Host cell entry of Middle East respiratory syndrome coronavirus after two-step, furin-mediated activation of the spike protein. *Proc Natl Acad Sci U S A*, 111(42):15214-9.
35. Huang C, Wang Y, Li X et al (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*, 395(10223):497-506.
36. Peiris M, Guan Y, Yuen K. (2005), *Severe acute respiratory syndrome*. 1st ed. Blackwell Pub, pp.: 72-76.
37. Liu J, Wu P, Gao F et al (2010). Novel immunodominant peptide presentation strategy: a featured HLA-A* 2402-restricted cytotoxic T-lymphocyte epitope stabilized by intrachain hydrogen bonds from severe acute respiratory syndrome coronavirus nucleocapsid protein. *J Virol*, 84(22):11849-57.
38. Keicho N, Itoyama S, Kashiwase K et al (2009). Association of human leukocyte antigen class II alleles with severe acute respiratory syndrome in the Vietnamese population. *Hum Immunol*, 70(7):527-31.
39. Xu Z, Shi L, Wang Y et al (2020). Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med*, 1;8(4):420-2.
40. Williams AE, Chambers RC. (2014). The mercurial nature of neutrophils: still an enigma in ARDS? *Am J Physiol Lung Cell Mol Physiol*, 306(3):L217-L30.
41. Cameron MJ, Bermejo-Martin JF, Danesh A et al (2008). Human immunopathogenesis of severe acute respiratory syndrome (SARS). *Virus Res*, 133(1):13-9.
42. Min C-K, Cheon S, Ha N-Y et al (2016). Comparative and kinetic analysis of viral shedding and immunological responses in MERS patients representing a broad spectrum of disease severity. *Sci Rep*, 6: 25359.
43. Ding Y, He L, Zhang Q, Huang Z et al (2004). Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. *The Journal of Pathology*, 203(2):622-30.
44. Xu J, Zhong S, Liu J et al (2005). Detection of severe acute respiratory syndrome coronavirus in the brain: potential role of the chemokine mig in pathogenesis. *Clin Infect Dis*, 41(8):1089-96.
45. Netland J, Meyerholz DK, Moore S et al (2008). Severe acute respiratory syndrome coronavirus infection causes neuronal death in the absence of encephalitis in mice transgenic for human ACE2. *J Virol*, 82(15):7264-75.
46. Li K, Wohlford-Lenane C, Perlman S et al (2016). Middle East respiratory syndrome coronavirus causes multiple organ damage and lethal disease in mice transgenic for human dipeptidyl peptidase 4. *J Infect Dis*, 213(5):712-22.
47. Li Y-C, Bai W-Z, Hirano N et al (2012). Coronavirus infection of rat dorsal root ganglia: ultrastructural characterization of viral replication, transfer, and the early response of satellite cells. *Virus Res*, 163(2):628-35.
48. Li YC, Bai WZ, Hirano N et al (2013). Neurotropic virus tracing suggests a membranous-coating-mediated mechanism

- for transsynaptic communication. *J Comp Neurol*, 521(1):203-12.
49. Wang D, Hu B, Hu C et al (2020). Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. *Jama*, doi: 10.1001.
50. Hoffmann M, Kleine-Weber H, Krueger N et al (2020). The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells. *BioRxiv*, doi.org/10.1101/2020.01.31.929042
51. Iwata-Yoshikawa N, Okamura T, Shimizu Y et al (2019). TMPRSS2 contributes to virus spread and immunopathology in the airways of murine models after coronavirus infection. *J Virol*, 93(6):e01815-18.
52. Zhou Y, Vedantham P, Lu K, et al (2015). Protease inhibitors targeting coronavirus and filovirus entry. *Antiviral Res*, 116:76-84.
53. Kuba K, Imai Y, Rao S et al (2005). A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus–induced lung injury. *Nat Med*, 11(8):875-9.
54. Zhang R, Pan Y, Fanelli V et al (2015). Mechanical stress and the induction of lung fibrosis via the midline signaling pathway. *Am J Respir Crit Care Med*, 192(3):315-23.
55. World Health Organization (WHO). Coronavirus disease (COVID-2019) situation reports. Available from: <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports>
56. Control CfD, Prevention. Interim pre-pandemic planning guidance: community strategy for pandemic influenza mitigation in the United States. Retrieved March. 2007;21:2007. Available from: https://www.cdc.gov/flu/pandemic-resources/pdf/community_mitigation-sm.pdf
57. Organization WH. Coronavirus disease 2019 (COVID-19) situation report–34. Geneva, Switzerland: World Health Organization; 2020. Available from: <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports>
58. Belouzard S, Millet JK, Licitra BN, Whittaker GR (2012). Mechanisms of coronavirus cell entry mediated by the viral spike protein. *Viruses*, 4(6):1011-33.