Coronavirus membrane fusion as a potential target for antiviral drug development

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Supplementary background information

The accompanying review article summarizes current understanding of coronavirus (CoV) membrane fusion, focusing on the two most studied viruses, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), and including recent findings on the newly emerged SARS-CoV-2. This supplementary file presents background information on CoV epidemiology and disease.

I. Coronavirus background

Coronaviruses (CoVs) are a family of enveloped viruses that infect a wide range of birds and mammals, causing respiratory and/or enteric tract infections (Fehr and Perlman, 2015). They are a highly successful group of viruses that have the potential for major impact on both human and animal health. Notably, the severity of coronavirus infection can vary widely; in humans, coronaviruses can cause a range of symptoms from a mild "common cold" to severe pneumonia and acute respiratory distress syndrome (ARDS). In animals, for example, feline enteric coronavirus (FECV) usually causes a mild or asymptomatic infection of cats, but can mutate leading to the development of a systemic and invariably fatal disease, feline infectious peritonitis (FIP) (Jaimes and Whittaker, 2018).

There are now seven well characterized human CoVs (Perlman and McIntosh, 2019; Zhou et al., 2020) **(Table 1)**. The first were recovered in the 1960s, and were shown to be morphologically similar to what is now the prototype CoV, avian infectious bronchitis, which was originally isolated in 1937. Two viruses, HCoV-229E and HCoV-OC43, were subsequently isolated from patients with the common cold, and from the 1960s until 2002-2003, when SARS-CoV emerged from its animal reservoir, no link was made between CoV and more severe respiratory illness in humans. Subsequent to the SARS epidemic, increased surveillance led to the discovery of two additional human coronaviruses, HCoV-HKU1 and HCoV-NL63, with HCoV-NL63 in particular linked to more severe disease, including croup (Perlman and McIntosh, 2019).

The coronavirus with the highest apparent case fatality rate for humans, MERS-CoV, was first detected in 2012-2013, and has since repeatedly been transferred from an animal reservoir to humans, causing single cases or small outbreaks, without epidemic spread. In contrast, the novel SARS-CoV-2 virus that emerged in late 2019 is transmitted rapidly from person to person, and over the course of three months has resulted in a pandemic. Human coronaviruses can therefore be described as either community-acquired respiratory (CAR) if they can sustain human-to-human transmission, or zoonotic if otherwise (Perlman and McIntosh, 2019). Currently, only SARS-CoV and MERS-CoV are classified as zoonotic whereas HCoV-229E and HCoV-OC43, HCoV-HKU1, HCoV-NL63 are classified as CAR. SARS-CoV-2 has now emerged as a new CAR CoV. Overall,

there is a wide range of coronavirus-induced disease, which has a major impact on public health, with no approved medical countermeasures, such as vaccines or antiviral drugs.

Table 1. Epidemiology profile and diseases signs of human coronaviruses				
1	HCoV-229E	CAR	Common cold	(Tyrrell and Bynoe, 1965)
2	HCoV-OC43	CAR	Common cold	(McIntosh et al., 1967)
3	SARS-CoV	Single zoonotic introduction > limited human-to-human transmission (eliminated)	Pneumonia	(Peiris et al., 2003)
4	HCoV-NL63	CAR	Common cold, croup	(Fouchier et al., 2004; Van Der Hoek et al., 2004)
5	HCoV-HKU1	CAR	Common cold	(Woo et al., 2005)
6	MERS-CoV	Recurrent zoonotic introductions	Pneumonia, renal disease	(Zaki et al., 2012)
7	SARS-CoV-2	Zoonotic introduction > recent pandemic CAR	Pneumonia, uncertain	(Zhou et al., 2020)

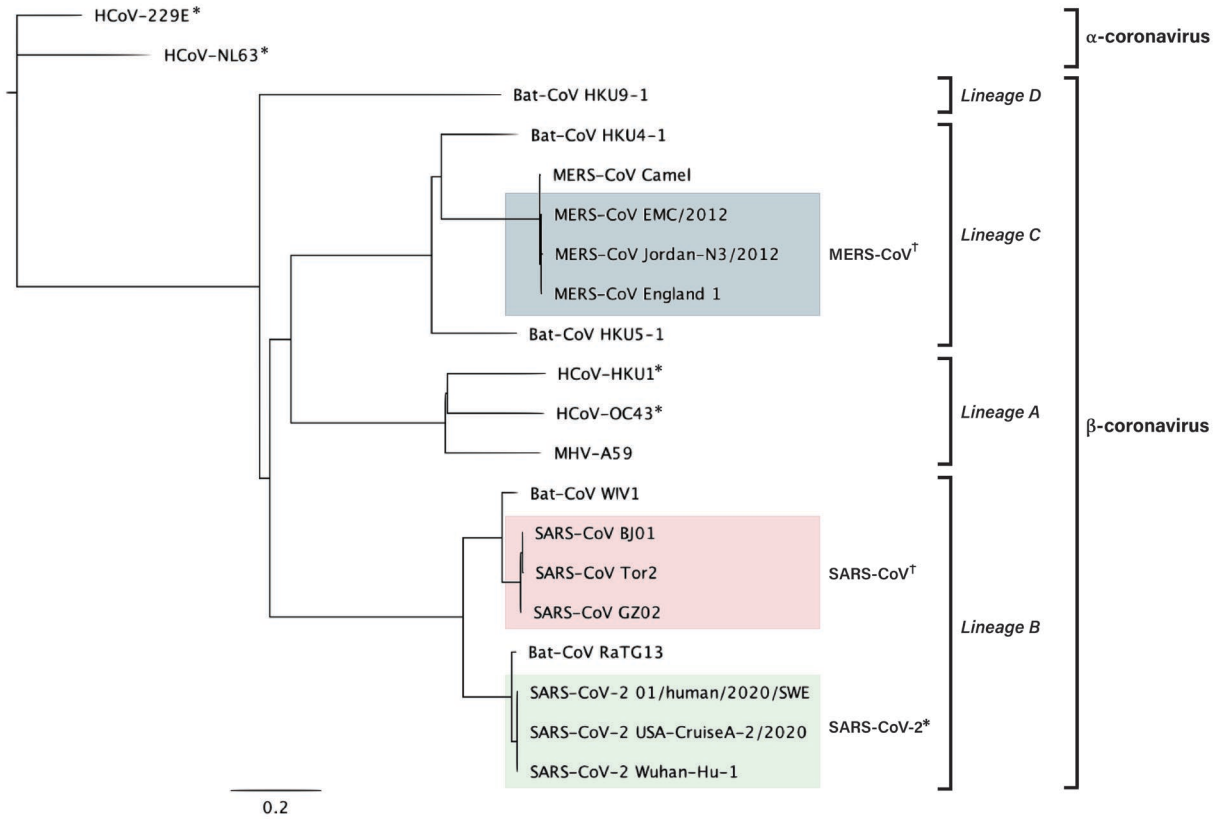
II. Coronaviruses as public health concerns

The 21st century has seen the rise of three pathogenic human coronaviruses: SARS-CoV, MERS-CoV and more recently, SARS-CoV-2. SARS-CoV was first reported in Southern China in the winter of 2002. Infected individuals usually exhibited mild respiratory symptoms that rapidly progressed into atypical pneumonia (Zhong et al., 2003). SARS-CoV then rapidly spread internationally, causing more than 8000 confirmed infections across 29 countries, with almost 800 deaths (case fatality rate: 9.6%) by July 2003 (Lam et al., 2004; Skowronski et al., 2005). Effective global health measures led by the World Health Organization, consisting of travel restrictions, patient isolation and raising public awareness contained the SARS outbreak, and by July 2003, WHO declared the end of the SARS outbreak, with the last reported case of SARS-identified in May 2004 (Mackenzie and Merianos, 2013).

About a decade later, the first MERS case was reported in Saudi Arabia; infected individuals also exhibited cold-like symptoms that developed into a fatal pneumonia (van Boheemen et al., 2012; Zaki et al., 2012). MERS has followed a pattern quite different from SARS, with recurrent introductions from animals to humans in the Middle East, occasionally carried to other countries by travelers, resulting in single cases or limited outbreaks. To date, approximately 2500 infected individuals have been identified in 27 countries, with more than 850 deaths (case fatality rate: 34.4%) (World Health Organization, 2020).

More recently, a new CoV, known as SARS-CoV-2, emerged in mainland China, causing a respiratory syndrome known as COVID-19 (Zhou et al., 2020). At the time of writing (4/3/2020), there have been nearly one million confirmed cases around the world, with approximately 30,000 deaths**.** Now classified as a pandemic, COVID-19 has highlighted the devastating impact of CoVs on human health and the need to develop effective strategies to prevent future outbreaks.

Surveillance studies conducted on SARS-CoV, MERS-CoV, and SARS-CoV-2 have found that these viruses originated in bats (Lau et al., 2005; Li et al., 2005; Wang et al., 2014; Zhou et al., 2020), which are the natural reservoir. Both SARS-CoV and MERS-CoV then transitioned into an intermediate host: civet cats and raccoon dogs for SARS-CoV (Guan et al., 2003; Li et al., 2006; Xu et al., 2009) and dromedary camels for MERS-CoV (Azhar et al., 2014; Mohd et al., 2016), and subsequently crossed into the human population (Cui et al., 2019). CoVs therefore possess immense zoonotic potential with devastating impacts on public health. This is especially important because novel SARS-CoV-like and MERS-CoV-like CoVs in bats have the capacity to infect human cells (Ge et al., 2013; Luo et al., 2018; Menachery et al., 2016; Wang et al., 2014). Furthermore, the novel swine acute diarrhea syndrome coronavirus (SADS-CoV), which recently devastated piglet populations in China, bears striking resemblance to the beginning of the SARS-CoV outbreak (Zhou et al., 2018). Our phylogenetic analysis highlights similarities among various human CoVs as well as their analogous animal viruses **(Figure 1),** emphasizing that there will likely be future CoV spillovers**.**



Supplementary Figure 1. **Coronavirus spike (S) protein phylogenetic tree**. The S protein sequence of representative human coronaviruses and analogous animal coronaviruses were aligned using Geneious Tree Builder neighbor-joining method. The outer brackets refer to the genus (α- or β-coronavirus), and the inner brackets to the lineage of β-coronavirus (A, B, C, or D).

The boxed strains indicate the viruses that have caused severe outbreaks in the human population and are of focus in this review (MERS-CoV, SARS-CoV, SARS-CoV-2). * indicate CAR CoV, ┼ indicate zoonotic CoV. The tree was generated using HCoV-229E as the outgroup and Jukes-Cantor as the genetic distance model. Amino acid sequence of the spike proteins was obtained from NCBI Genbank based on the following: HCoV-229E (AAK32188.1), HCoV-NL63 (AGT51331), Bat-CoV HKU9-1 (NC_009021.1), Bat-CoV HKU4-1 (AY597011.2), MERS-CoV Camel (KJ650295.1), MERS-CoV EMC/2012 (JX869059.2), MERS-CoV Jordan-N3/2012 (KC776174.1), MERS-CoV England 1 (KC164505.2), Bat-CoV HKU5-1 (EF065509.1), HCoV-HKU1 (AY597011.2), HCoV-OC43 (KF963244.1), MHV-A59 (M18379.1), Bat-CoV WIVI (KC881007.1), SARS-CoV BJ01 (AY278488.2), SARS-CoV Tor2 (NC_004718.3), SARS-CoV GZ02 (AY390556.1), Bat-CoV RaTG13 (MN996532.1), SARS-CoV-2 01/human/2020/SWE (MT093571.1), SARS-CoV-2 USA-CruiseA-2/2020 (MT159718.1), and SARS-CoV-2 Wuhan-Hu-1 (MN908947.3).

References

Azhar, E.I., El-Kafrawy, S.A., Farraj, S.A., Hassan, A.M., Al-Saeed, M.S., Hashem, A.M., Madani, T.A., 2014. Evidence for Camel-to-Human Transmission of MERS Coronavirus. N. Engl. J. Med. 370, 2499–2505. https://doi.org/10.1056/NEJMoa1401505

Cui, J., Li, F., Shi, Z.-L., 2019. Origin and evolution of pathogenic coronaviruses. Nat. Rev. Microbiol. 17, 181–192. https://doi.org/10.1038/s41579-018-0118-9

Fehr, A.R., Perlman, S., 2015. Coronaviruses: An overview of their replication and pathogenesis. Coronaviruses Methods Protoc. 1282, 1–23. https://doi.org/10.1007/978-1-4939-2438-7_1

Fouchier, R.A.M., Hartwig, N.G., Bestebroer, T.M., Niemeyer, B., De Jong, J.C., Simon, J.H., Osterhaus, A.D.M.E., 2004. A previously undescribed coronavirus associated with respiratory disease in humans. Proc. Natl. Acad. Sci. U. S. A. 101, 6212–6216. https://doi.org/10.1073/pnas.0400762101

Ge, X.-Y., Li, J.-L., Yang, X.-L., Chmura, A.A., Zhu, G., Epstein, J.H., Mazet, J.K., Hu, B., Zhang, W., Peng, C., Zhang, Y.-J., Luo, C.-M., Tan, B., Wang, N., Zhu, Y., Crameri, G., Zhang, S.-Y., Wang, L.-F., Daszak, P., Shi, Z.-L., 2013. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature 503, 535–538. https://doi.org/10.1038/nature12711

Guan, Y., Zheng, B.J., He, Y.Q., Liu, X.L., Zhuang, Z.X., Cheung, C.L., Luo, S.W., Li, P.H., Zhang, L.J., Guan, Y.J., Butt, K.M., Wong, K.L., Chan, K.W., Lim, W., Shortridge, K.F., Yuen, K.Y., Peiris, J.S.M., Poon, L.L.M., 2003. Isolation and Characterization of Viruses Related to the SARS Coronavirus from Animals in Southern China. Science (80-.). 302, 276–278. https://doi.org/10.1126/science.1087139

Jaimes, J.A., Whittaker, G.R., 2018. Feline coronavirus: Insights into viral pathogenesis based on the spike protein structure and function. Virology 517, 108–121. https://doi.org/10.1016/J.VIROL.2017.12.027

Lam, C.W.K., Chan, M.H.M., Wong, C.K., 2004. Severe acute respiratory syndrome: clinical and laboratory manifestations. Clin. Biochem. Rev. 25, 121–32.

Lau, S.K.P., Woo, P.C.Y., Li, K.S.M., Huang, Y., Tsoi, H.-W., Wong, B.H.L., Wong, S.S.Y., Leung, S.-Y., Chan, K.-H., Yuen, K.-Y., 2005. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. Proc. Natl. Acad. Sci. U. S. A. 102, 14040–5. https://doi.org/10.1073/pnas.0506735102

Li, W., Shi, Z., Yu, M., Ren, W., Smith, C., Epstein, J.H., Wang, H., Crameri, G., Hu, Z., Zhang, H., Zhang, J., McEachern, J., Field, H., Daszak, P., Eaton, B.T., Zhang, S., Wang, L.-F., 2005. Bats Are Natural Reservoirs of SARS-Like Coronaviruses. Science (80-.). 310, 676–679. https://doi.org/10.1126/science.1118391

Li, W., Wong, S.-K., Li, F., Kuhn, J.H., Huang, I.-C., Choe, H., Farzan, M., 2006. Animal origins of the severe acute respiratory syndrome coronavirus: insight from ACE2-S-protein interactions. J. Virol. 80, 4211–9. https://doi.org/10.1128/JVI.80.9.4211-4219.2006

Luo, C.-M., Wang, N., Yang, X.-L., Liu, H.-Z., Zhang, W., Li, B., Hu, B., Peng, C., Geng, Q.-B., Zhu, G.-J., Li, F., Shi, Z.-L., 2018. Discovery of Novel Bat Coronaviruses in South China That Use the Same Receptor as Middle East Respiratory Syndrome Coronavirus. J. Virol. 92, e00116-18. https://doi.org/10.1128/JVI.00116-18

Mackenzie, J.S., Merianos, A., 2013. The legacies of SARS - international preparedness and readiness to respond to future threats in the Western Pacific Region. West. Pacific Surveill. response J. WPSAR 4, 4–8. https://doi.org/10.5365/WPSAR.2013.4.3-004

McIntosh, K., Dees, J.H., Becker, W.B., Kapikian, A.Z., Chanock, R.M., 1967. Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. Proc. Natl. Acad. Sci. U. S. A. 57, 933–940. https://doi.org/10.1073/pnas.57.4.933

Menachery, V.D., Yount, B.L., Sims, A.C., Debbink, K., Agnihothram, S.S., Gralinski, L.E., Graham, R.L., Scobey, T., Plante, J.A., Royal, S.R., Swanstrom, J., Sheahan, T.P., Pickles, R.J., Corti, D., Randell, S.H., Lanzavecchia, A., Marasco, W.A., Baric, R.S., 2016. SARS-like WIV1-CoV poised for human emergence. Proc. Natl. Acad. Sci. U. S. A. 113, 3048–53. https://doi.org/10.1073/pnas.1517719113

Mohd, H.A., Al-Tawfiq, J.A., Memish, Z.A., 2016. Middle East Respiratory Syndrome Coronavirus (MERS-CoV) origin and animal reservoir. Virol. J. 13, 87. https://doi.org/10.1186/s12985-016-0544-0

Peiris, J.S.M., Lai, S.T., Poon, L.L.M., Guan, Y., Yam, L.Y.C., Lim, W., Nicholls, J., Yee, W.K.S., Yan, W.W., Cheung, M.T., Cheng, V.C.C., Chan, K.H., Tsang, D.N.C., Yung, R.W.H., Ng, T.K., Yuen, K.Y., 2003. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 361, 1319–1325. https://doi.org/10.1016/S0140-6736(03)13077-2

Perlman, S., McIntosh, K., 2019. Coronaviruses, Including Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome, in: Principles and Practice of Infectious Diseases. pp. 2072-.

Skowronski, D.M., Astell, C., Brunham, R.C., Low, D.E., Petric, M., Roper, R.L., Talbot, P.J., Tam, T., Babiuk, L., 2005. Severe Acute Respiratory Syndrome (SARS): A Year in Review. Annu. Rev. Med. 56, 357–381. https://doi.org/10.1146/annurev.med.56.091103.134135

Tyrrell, D.A.J., Bynoe, M.L., 1965. Cultivation of a Novel Type of Common-cold Virus in Organ Cultures. Br. Med. J. 1, 1467–1470. https://doi.org/10.1136/bmj.1.5448.1467

van Boheemen, S., de Graaf, M., Lauber, C., Bestebroer, T.M., Stalin Raj, V., Moh Zaki, A., M E Osterhaus, A.D., Haagmans, B.L., Gorbalenya, A.E., Snijder, E.J., M Fouchier, R.A., Fakeeh Hospital, S., Arabia, S., Michael Buchmeier, E., 2012. Genomic Characterization of a Newly Discovered Coronavirus Associated with Acute Respiratory Distress Syndrome in Humans. https://doi.org/10.1128/mBio.00473-12

Van Der Hoek, L., Pyrc, K., Jebbink, M.F., Vermeulen-Oost, W., Berkhout, R.J.M., Wolthers, K.C., Wertheim-Van Dillen, P.M.E., Kaandorp, J., Spaargaren, J., Berkhout, B., 2004. Identification of a new human coronavirus. Nat. Med. 10, 368–373. https://doi.org/10.1038/nm1024

Wang, Q., Qi, J., Yuan, Y., Xuan, Y., Han, P., Wan, Y., Ji, W., Li, Y., Wu, Y., Wang, J., Iwamoto, A., Woo, P.C.Y., Yuen, K.-Y., Yan, J., Lu, G., Gao, G.F., 2014. Bat Origins of MERS-CoV Supported by Bat Coronavirus HKU4 Usage of Human Receptor CD26. Cell Host Microbe 16, 328–337. https://doi.org/10.1016/J.CHOM.2014.08.009

Woo, P.C.Y., Lau, S.K.P., Chu, C. -m., Chan, K. -h., Tsoi, H. -w., Huang, Y., Wong, B.H.L., Poon, R.W.S., Cai, J.J., Luk, W. -k., Poon, L.L.M., Wong, S.S.Y., Guan, Y., Peiris, J.S.M., Yuen, K. -y., 2005. Characterization and Complete Genome Sequence of a Novel Coronavirus, Coronavirus HKU1, from Patients with Pneumonia. J. Virol. 79, 884–895. https://doi.org/10.1128/jvi.79.2.884-895.2005

World Health Organization, 2020. Middle East respiratory syndrome coronavirus (MERS-CoV). WHO.

Xu, L., Zhang, Y., Liu, Y., Chen, Z., Deng, H., Ma, Z., Wang, H., Hu, Z., Deng, F., 2009. Angiotensin-converting enzyme 2 (ACE2) from raccoon dog can serve as an efficient receptor for the spike protein of severe acute respiratory syndrome coronavirus. J. Gen. Virol. 90, 2695–2703. https://doi.org/10.1099/vir.0.013490-0

Zaki, A.M., van Boheemen, S., Bestebroer, T.M., Osterhaus, A.D.M.E., Fouchier, R.A.M., 2012. Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia. N. Engl. J. Med. 367, 1814–1820. https://doi.org/10.1056/NEJMoa1211721

Zhong, N., Zheng, B., Li, Y., Poon, L., Xie, Z., Chan, K., Li, P., Tan, S., Chang, Q., Xie, J., Liu, X., Xu, J., Li, D., Yuen, K., Peiris, J., Guan, Y., 2003. Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. Lancet 362, 1353–1358. https://doi.org/10.1016/S0140-6736(03)14630-2

Zhou, P., Fan, H., Lan, T., Yang, X. Lou, Shi, W.F., Zhang, W., Zhu, Y., Zhang, Y.W., Xie, Q.M., Mani, S., Zheng, X.S., Li, B., Li, J.M., Guo, H., Pei, G.Q., An, X.P., Chen, J.W., Zhou, L., Mai, K.J., Wu, Z.X., Li, D., Anderson, D.E., Zhang, L.B., Li, S.Y., Mi, Z.Q., He, T.T., Cong, F., Guo, P.J., Huang, R., Luo, Y., Liu, X.L., Chen, J., Huang, Y., Sun, Q., Zhang, X.L.L., Wang, Y.Y., Xing, S.Z., Chen, Y.S., Sun, Y., Li, J., Daszak, P., Wang, L.F., Shi, Z.L., Tong, Y.G., Ma, J.Y., 2018. Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. Nature 556, 255–259. https://doi.org/10.1038/s41586-018-0010-9

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., Chen, H.-D., Chen, J., Luo, Y., Guo, H., Jiang, R.-D., Liu, M.-Q., Chen, Y., Shen, X.-R., Wang, X., Zheng, X.-S., Zhao, K., Chen, Q.-J., Deng, F., Liu, L.-L., Yan, B., Zhan, F.-X., Wang, Y.-Y., Xiao, G.-F., Shi, Z.-L., 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579, 270–273. https://doi.org/10.1038/s41586-020-2012-7