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SKILLFUL ACQUISITIONS THAT ARMS SARS-CoV-2

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ABSTRACT: The pandemic which has spread throughout continents makes you wonder, what are the additional acquisitions of the novel coronavirus that makes it skillfully effective in human to human transmission. Some of the skillful acquisitions like mutations in the receptor-binding domain of SARS-CoV-2, incorporation of polybasic furin cleavage site and addition of O-linked glycans may explain in parts, the infectiousness and high transmissibility of SARS-CoV-2 in humans.

Key Words: SARS-CoV-2, COVID-19, coronavirus, pandemic, mutations.

INTRODUCTION:

China reported cases of pneumonia of unknown etiology in Wuhan to WHO on 31st December 2019. A novel coronavirus was identified as the cause of the unusual pneumonia (Zang and Holmes, 2020). World Health Organization (WHO) named the novel coronavirus as SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) and the disease as coronavirus disease 2019 (COVID-19) (Gobalenya et al., 2020). On 30th January 2020, WHO declared the corona virus outbreak as Public Health Emergency of International Concern (PHEIC) (Chan et al., 2020, Mahtani et al., 2020, WHO, second meeting 2020) and pandemic on 11th March 2020 (WHO, 2020).

SARS-CoV-2 is the seventh known coronavirus to infect people, after 229E, NL63, OC43, HKU1, MERS-CoV and the original SARS-CoV (Zhu et al., 2020). SARS-CoV-2 is a positive sense single stranded RNA virus (*GISAID Epiflu, 2020*). Based on the sense or polarity of the RNA, the single stranded RNA viruses are classified

as positive and negative. The positive-sense viral RNA genome can serve as messenger RNA (mRNA) and can be directly translated into proteins in the host cell. SARS-CoV-2 is closely related to both SARS-CoV and MERS-CoV. MERS-CoV was not quite adapted to human transmission (Sabir et al., 2016) but SARS-CoV-2 is more infectious, armed with skillful adaptations for human-to-human transmission, spreading across continents, in susceptible populations.

Zoonotic diseases are often caused by harmful germs carried by animals and passed on to humans. The WHO considers bats to be the most likely natural reservoir of SARS-CoV-2 (WHO, 24 February 2020). Previously in MERS-CoV, and SARS-CoV, camels and civets acted as intermediate hosts. There being distinct ecological difference between bats and humans, an intermediate animal is thought to be involved in the introduction of SARS-CoV-2 to humans (WHO, 11 February 2020). This concept took precedent when on 7th February 2020, it was announced that researchers from Guangzhou had discovered a Pangolin sample with a viral nucleic acid sequence "99% identical" to SARS-CoV-2 (Cyranoski, 2020).

Past experience also suggests the evolution of coronaviruses in animal hosts, both in reservoirs and intermediates. Unable to infect humans directly through bats, SARS-CoV-2 probably had to go through an intermediate animal to further mutate, so that it could be transmitted to humans (Zhang and Holmes, 2020). Some key acquisitions must have armed SARS-CoV-2 to jump species boundaries and adapt to new hosts. The virus must have acquired them prior to its first detection in December 2019.

Each SARS-CoV-2 virion is approximately 50–200 nanometer in diameter (Chen et al., 2020). It has four structural proteins, known as the S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins. The attachment of the virus to the host cell surface receptor and ensuing fusion between viral and host cell membrane for the virus entry is facilitated by the S protein (Kirchdoerfer et al., 2016). The M protein is the most abundant structural protein and it defines the shape of the viral envelope (Neuman et al., 2011). The E protein is the smallest of the major structural proteins (Venkatagopalan et al., 2015). N proteins bind to the CoV RNA genome, also called the nucleocapsid (de Haan and Rottier, 2005). The RNA sequence of SARS-CoV-2 is approximately 30,000 bases in length (GISAID *Epiflu*, 2020).

The viral envelope-anchored spike protein S has S1 and S2 subunits. The receptor binding domain (RBD) in the S1 subunit first binds with the host receptor then the S2 subunit facilitates the fusion of viral and host membrane (Li et al., 2005). The host receptor for MERS-CoV is Dipeptidyl peptidase 4 (DPP4) and for SARS-CoV, it is angiotensin converting enzyme 2 (ACE 2). The RBD of SARS-CoV-2 also recognizes and binds with ACE 2 receptor of the host (Zhou et al., 2020, Lu et al., 2020). The recognition of host receptor by coronaviruses is the first step for infecting host cell (Li, 2015, Fehr and Perlman, 2015). The RBD of spike protein has receptor binding motifs (RBM) which directly binds to the ACE 2 of the host. The binding affinity reflects the infectivity and transmissibility of the virus (Li et al., 2005, Wan et al., 2020).

ACE2 is a type I membrane protein expressed in lungs, heart, kidneys, and intestine (Zhao et al., 2020, Zhang and Holmes 2020, Donoghue, 2020). The primary function of ACE 2 is maturation of angiotensin. Angiotensin is a peptide hormone that controls vasoconstriction and blood pressure. Decreased expression of ACE2 is associated with cardiovascular diseases (Crackower et al., 2002, Zisman et al., 2003, Raizada and Ferreira, 2007). Both sequence and structural comparisons suggest that the SARS-CoV-2 RBD is well suited for binding to the human ACE2 receptor that was also utilized by SARS-CoV (Wrap et al., 2020)

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Three coronaviruses have crossed the species barrier to cause deadly pneumonia in humans since the beginning of the 21st century, MERS-CoV, SARS-CoV and very recently SARS-CoV-2. The skillful acquisitions described here may explain in parts, the infectiousness and high transmissibility of SARS-CoV-2 in humans.

Mutations in the receptor-binding domain of SARS-CoV-2

The receptor-binding domain (RBD) in the spike protein is the most variable part of the coronavirus genome (Zhou et al., 2020). In SARS-CoV-like viruses, six RBD amino acids are important for binding to ACE2 receptors and determining the host range. SARS-CoV-2 seems to have an RBD that binds with high affinity to ACE2 receptors of humans (Wan et al., 2020). It has been shown that five of these six residues differ between SARS-CoV-2 and SARS-CoV.

Incorporation of Polybasic furin cleavage site

The trimeric transmembrane spike (S) glycoprotein of SARS-CoV-2 facilitates the entry of virus particles into the host cell. The S protein contains two functional domains: a receptor binding domain (RBD), and a second domain

has a sequence that mediates the fusion of viral and cell membranes. The viral transmembrane spike glycoprotein is cleaved by proteases from host cell to enable the exposure of fusion sequences needed for cell entry (Madu et al., 2009).

The second notable feature of SARS-CoV-2 is incorporation of a polybasic cleavage site (RRAR) at the junction of S1 and S2, the two subunits of the spike (Walls et al., 2020). The significance of inclusion of a polybasic cleavage site in SARS-CoV-2 is unknown. However, this allows effective cleavage by furin and other proteases and has a role in determining viral infectivity and host range (Nao et al., 2016). Experiments with SARS-CoV have shown that insertion of a furin cleavage site at the S1–S2 junction enhances cell–cell fusion without affecting viral entry (Follis et al., 2006). However, the highly related bat CoV RaTG-13 does not have the furin cleavage site. Furin proteases are plentiful in the respiratory tract, probably SARS-CoV-2 S glycoprotein is cleaved upon exit from epithelial cells. This consequently enables the virus to efficiently infect other cells. SARS-CoV-2 is unique among known beta-coronaviruses as it has incorporated a polybasic cleavage site in the spike (S) glycoprotein. It is a property known to enhance pathogenicity and transmissibility in other viruses (Andersen et al., 2020).

Addition of O-linked glycans

In transmembrane spike (S) glycoprotein of SARS-CoV-2, a leading proline is inserted at the polybasic cleavage site (RRAR) thus, the inserted sequence is PRRA. This has resulted in the addition of an O-linked glycan at the cleavage site. Addition of an O-linked glycan is an unique property of SARS-CoV-2 and its function is unclear. Bagdonaite and Wandall, 2018 had suggested that it could result in a 'mucin-like domain'. Several viruses use them as glycan shields for immune evasion.

CONCLUSION:

Skillful acquisitions that has armed the SARS-CoV-2 has harmed mankind extensively. Thus it is reasonable to questions the origin of SARS-CoV-2, its adaptations and zoonotic events that have allowed this novel coronavirus to jump species boundaries and infect humans so productively. SARS-CoV-2 and bat SARS-CoV-like coronaviruses being similar, it is likely that bats serve as reservoir hosts for its progenitor. SARS-CoV-2 probably had to go through an intermediate animal to further mutate, so that it could be transmitted to humans. The adaptations like mutations in the receptor-binding domain of SARS-CoV-2, incorporation of Polybasic furin cleavage site and addition of an O-linked glycan partly explains the infectiousness and efficient transmissibility of SARS-CoV-2 in humans.

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