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The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade

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ABSTRACT

In 2019, a new coronavirus (2019-nCoV) infecting Humans has emerged in Wuhan, China. Its genome has been sequenced and the genomic information promptly released. Despite a high similarity with the genome sequence of SARS-CoV and SARS-like CoVs, we identified a peculiar furin-like cleavage site in the Spike protein of the 2019-nCoV, lacking in the other SARS-like CoVs. In this article, we discuss the possible functional consequences of this cleavage site in the viral cycle, pathogenicity and its potential implication in the development of antivirals.

Human coronaviruses (CoV) are enveloped positive-stranded RNA viruses belonging to the order Nidovirales, and are mostly responsible for upper respiratory and digestive tract infections. Among them SARS-CoV and MERS-CoV that spread in 2002 and 2013 respectively, have been associated with severe human illnesses, such as severe pneumonia and bronchiolitis, and even meningitis in more vulnerable populations (de Wit et al., 2016). In December 2019, a new CoV (2019-nCoV) has been detected in the city of Wuhan, and this emerging viral infection was associated with severe human respiratory disease with a ~2–3% fatality rate (Li et al., 2020). The virus that was presumed to have initially been transmitted from an animal reservoir to humans possibly via an amplifying host. However human-to-human transmission has been reported, leading to a sustained epidemic spread with >31,000 confirmed human infections, including >640 deaths, reported by the WHO in early February 2020. The estimated effective reproductive number (R) value of ~2.90 (95%: 2.32–3.63) at the beginning of the outbreak raises the possibility of a pandemic (Zhao et al., 2020). This prompted WHO to declare it as a Public Health Emergency of International Concern. This is especially relevant because so far there are no specific antiviral treatments available or vaccine. Based on its genome sequence, 2019-nCoV belongs to lineage b of Betacoronavirus (Fig. 1A), which also includes the SARS-CoV and bat CoV ZXC21, the latter and CoV ZC45 being the closest to 2019-nCoV. 2019-nCoV shares ~76% amino acid sequence identity in the Spike (S)-protein sequence with SARS-CoV and 80% with CoV ZXC21 (Chan et al., 2020). In this article, we focus on a specific furin-like protease recognition pattern present in the vicinity of one of the maturation sites of the S protein (Fig. 1B) that may have significant functional implications for virus entry.

The proprotein convertases (PCs; genes PCSKs) constitute a family of nine serine secretary proteases that regulate various biological processes in both healthy and disease states (Seidah and Prat, 2012). By proteolysis, PCs are responsible for the activation of a wide variety of precursor proteins, such as growth factors, hormones, receptors and adhesion molecules, as well as cell surface glycoproteins of infectious viruses (Seidah and Chretien, 1999) (Table 1). Seven PCs cleave precursor proteins at specific single or paired basic amino acids (aa) within the motif (R/K)-(2X)n-(R/K), where n = 0, 1, 2, or 3 spacer aa (Seidah and Chretien, 1999). Because of their role in the processing of many critical cell surface proteins PCs, especially furin, have been implicated in viral infections. They have the potential to cleave specifically viral envelope glycoproteins, thereby enhancing viral fusion with host cell membranes (Izaguirre, 2019; Moulard and Decroly, 2000). In the case of human-infecting coronaviruses such as HCoV-OC43 (Le Coupanc et al., 2015), MERS-CoV (Millet and Whittaker, 2014), and HKU1 (Chan et al., 2008) the spike protein has been demonstrated to be cleaved at an S1/S2 cleavage site (Fig. 2) generating the S1 and S2 subunits. The above three viruses display the canonical (R/K)-(2X)n-(R/K)↓ motif (Table 1). Additionally, it has been demonstrated that variation around the viral envelope glycoprotein cleavage site plays a role in cellular tropism and pathogenesis. For instance, the pathogenesis of some CoV
has been previously related to the presence of a furin-like cleavage site in the S-protein sequence. For example, the insertion of a similar cleavage site in the infectious bronchitis virus (IBV) S-protein results in higher pathogenicity, pronounced neural symptoms and neurotropism in infected chickens (Cheng et al., 2019).

Similarly, in the case of influenza virus, low-pathogenicity forms of influenza virus contain a single basic residue at the cleavage site, which is cleaved by trypsin-like proteases and the tissue distribution of the activating protease(s) typically restricts infections to the respiratory and/or intestinal organs (Sun et al., 2010). Conversely, the highly pathogenic forms of influenza have a furin-like cleavage site cleaved by different cellular proteases, including furin, which are expressed in a wide variety of cell types allowing a widening of the cell tropism of the virus (Kido et al., 2012). Furthermore the insertion of a multibasic motif REKRRK at the H5N1 hemagglutinin HA cleavage site was likely associated with the hyper-virulence of the virus during the Hong Kong 1997 outbreak (Claas et al., 1998). This motif exhibits the critical Arg at P1 and basic residues at P2 and P4, as well as P6 and P8 and an aliphatic Leu at P2' positions (Table 1) (Schechter and Berger, 1968), typical of a furin-like cleavage specificity (Braun and Sauter, 2019; Izaguirre, 2019; Seidah and Prat, 2012).

The coronavirus S-protein is the structural protein responsible for the crown-like shape of the CoV viral particles, from which the original name “coronavirus” was coined. The ~1200 aa long S-protein contains several conserved domains and motifs

**Table 1**

Comparative sequences of envelope protein cleavage site(s) in coronaviruses (above) and in other RNA viruses (below). Empty boxes: no consensus motif detected.

| Coronavirus    | S1/S2, site 1 | S1/S2, site 2 | S2' |
|----------------|---------------|---------------|-----|
| 2019-nCoV      | SRRRRR | IATIINS | SKFSRRR | SF |
| CoV-ZXC21      | TASILRTI | IATIINS | SKFSRRR | SF |
| Bat-AC45       | TASILRTI | IATIINS | SKFSRRR | SF |
| SARS-CoV       | TSVLKLRTI | IATIINS | LEKFSRRR | SF |
| BM48-31        | SSTLRLQQG | IATIINS | LEKFSRRR | SF |
| HKU9-1         | ADILFRQLQG | VNTLVVL | GATYRSLA|
| MERS-CoV       | TEFSCRHVVG | GSRARRSA | |
| HKU1           | SRRRRR | SISA | CSRRRR | SF |
| HCoV-OC43      | KNRKRR | GASTT | SKASSRSLA|
| HCoV-229E      | IAQQPR | MVSGYD | SRVACSRLA|
| HCoV-NL63      | IPYRRR | NSIDN | SRIAGSRLA|

**Fig. 1.** Characterization of an nCoV-peculiar sequence at the S1/S2 cleavage site in the S-protein sequence, compared SARS-like CoV. (A) Phylogenetic tree of selected coronaviruses from genera alphacoronavirus (α-Cov) and betacoronavirus (β-Cov), lineages a, b, c and d: 2019-nCoV (NC_045512.2), CoV-ZXC21 (MG772934), SARS-CoV (NC_004718.3), SARS-like BM4821 (MG772934), HCoV-Oc43 (AY391777), HKU9-1 (EF065513), HCoV-NL63 (KF530114.1), HCoV229E (KF514433.1), MERS-CoV (NC019843.3), HKU1 (NC_006577.2). The phylogenetic tree was obtained on the Orf1ab amino acid sequence using the Maximum Likelihood method by Mega X software. Red asterisks indicate the presence of a canonical furin-like cleavage motif at site 1; (B) Alignment of the coding and amino acid sequences of the S-protein from CoV-ZXC21 and 2019-nCoV at the S1/S2 site. The 2019-nCoV-specific sequence is in bold. The sequence of CoV-ZXC21 S-protein at this position is representative of the sequence of the other betacoronaviruses belonging to lineage b, except the one of 2019-nCoV. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
Fig. 2. Schematic representation of the human 2019-nCoV S-protein with a focus on the putative maturation sites. The domains were previously characterized in SARS-CoV and MERS-CoV: Signal peptide (SP), N-terminal domain (NTD), receptor-binding domain (RBD), fusion peptide (FP), internal fusion peptide (IFP), heptad repeat 1/2 (HR1/2), and the transmembrane domain (TM). The SP, S1/S2 and S2′ cleavage sites are indicated by arrows. The sequence of different CoV S1/S2 and S2′ cleavage sites were aligned using Multalin webserver (http://multalin.toulouse.inra.fr/multalin/) with manual adjustments and the figure prepared using ESPript 3 (http://esprit.ibcp.fr/ES Pript/ESPript/) presenting the secondary structure of SARS-CoV S-protein at the bottom of the alignment (PDB 5X58)( Yuan et al., 2017). Insertion of furin like cleavage site is surrounded by a black frame. Red asterisks indicate the presence of a canonical furin-like cleavage motif at the S1/S2 site. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
exposed P
innovation programme under grant agreement No 871029. This furin-like cleavage site, is supposed to be cleaved during virus egress (Mille and Whittaker, 2014) for sustained inhibition, deserve to be rapidly tested to assess their some toxicity. Accordingly, it is likely that such small molecule in-
hibitor form a complex with furin (Dahms et al., 2017). As furin-like dideoxystreptamine-derived inhibitor, where two molecules of the in-
hibitors of furin-like enzymes may contribute to inhibiting virus propagation.

A variety of approaches have been proposed to inhibit furin activity to limit tumour growth, viral and bacterial infection. Thus, a variant of the naturally occurring serine protease inhibitor α-antitrypsin harbouring a consensus furin cleavage, called α1-antitrypsin Portland (α1-PDX), inhibits furin and prevents the processing of HIV-1 Env (Anderson et al., 1993). The addition of a chloromethylketone (CMK) moiety to the C-terminus of a polybasic cleavage motif and a decanoyl group at the N-terminus to favour cell penetration (de-RVKR-cmk) irreversibly blocked the enzymatic activity of furin, PC7, PC5, PACE4 and PC7 (Decroly et al., 1996; Garten et al., 1994). Finally, the eluci-
dation of the crystal structure of furin resulted in the design of a 2,5-
dideoxystreptamine-derived inhibitor, where two molecules of the in-
hibitor form a complex with furin (Dahms et al., 2017). As furin-like enzymes are involved in a multitude of cellular processes, one im-
portant issue would be to avoid systemic inhibition that may result in some toxicity. Accordingly, it is likely that such small molecule in-
hibitors, or other more potent orally active ones, possibly delivered by inhalation and exhibiting a slow dissociation rate from furin to allow for sustained inhibition, deserve to be rapidly tested to assess their antiviral effect against 2019-nCoV.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2020.104742.

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