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

B. Coutard, C. Valle, X. de Lamballerie, B. Canard, N.G. Seidah, E. Decroly

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. 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.
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 REKRKRGL 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 nomenclature (Schechter and Berger, 1968)), typical of a furin-like cleavage specificity (Braun and Sauter, 2019; Izaguirre, 2019; Seidah and Prat, 2012).

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.

<table>
<thead>
<tr>
<th>Coronavirus</th>
<th>S1/S2, site 1</th>
<th>S1/S2, site 2</th>
<th>S2’</th>
</tr>
</thead>
<tbody>
<tr>
<td>2019-nCoV</td>
<td>SPRRARQVAS</td>
<td>IATJINS</td>
<td>SKFRKRSTF</td>
</tr>
<tr>
<td>CoV-ZXC21</td>
<td>TASSLIRGQQ</td>
<td>IATJINS</td>
<td>SKFRKRSTF</td>
</tr>
<tr>
<td>Bat-AC58</td>
<td>TASSLIRGQQ</td>
<td>IATJINS</td>
<td>SKFRKRSTF</td>
</tr>
<tr>
<td>SARS-CoV</td>
<td>TVEEILGQQ</td>
<td>IATJINS</td>
<td>LEFRKRSTF</td>
</tr>
<tr>
<td>BM48-31</td>
<td>SSTDDLGEDDD</td>
<td>IATJINS</td>
<td>LFPRKRSTF</td>
</tr>
<tr>
<td>HKU1-1</td>
<td>ADILLSLGLSV</td>
<td>GATTVRISA</td>
<td>PRISSSIS</td>
</tr>
<tr>
<td>MERS-CoV</td>
<td>TDFRCKRPVC</td>
<td>GSRARSISA</td>
<td>CUGSSSISR</td>
</tr>
<tr>
<td>HKU1</td>
<td>SRRRSISA</td>
<td>CUGSSSISR</td>
<td>SIAASAS</td>
</tr>
<tr>
<td>HCoV-OC43</td>
<td>KRRSRSGASTT</td>
<td>SKASASRSA</td>
<td>RSAASAS</td>
</tr>
<tr>
<td>HCoV-229E</td>
<td>TAVGPRNVSVD</td>
<td>SAVASRSA</td>
<td>RSAASAS</td>
</tr>
<tr>
<td>HCoV-NL63</td>
<td>IPVFRNHSSLDN</td>
<td>SIAASAS</td>
<td>RSAASAS</td>
</tr>
</tbody>
</table>

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), HKU1-1 (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.)
Protein must likely be cleaved at both S1/S2 and S2′ surfaces of S1/ACE2 implicates 14 aa in the S1 of SARS-CoV (Li et al., 2003). Notably, the IFPs of the 2019-nCoV and SARS-CoV are identical, displaying characteristics of viral fusion peptides (Fig. 2). While the molecular mechanism involved in virus entry, the enzymes of the target cells should regulate cellular tropism and viral pathogenesis. In the case of the 2019-nCoV S-protein, the protease(s) involved in this process have not yet been conclusively identified. Based on the 2019-nCoV S2′ sequence and the above arguments, we propose that one or more furin-like enzymes would cleave the S2′ site at KR↓SF. In contrast to the S2′, the first cleavage between the RBD and the FP (S1/S2 cleavage site, Fig. 2) has been extensively studied for many CoVs (Lu et al., 2015). Interestingly the S1/S2 processing site exhibits different motifs among coronaviruses (Fig. 2, site 1 & site 2), with many of them displaying cleavage after a basic residue. It is thus likely that the priming process is ensured by different host cell proteases depending on the sequence of the S1/S2 cleavage site. Accordingly the MERS-CoV S-protein, which contains a RSV↓SF motif is cleaved during virus egress, probably by furin (Mille and Whitaker, 2014). Conversely the S-protein of SARS-CoV remains largely uncleaved after biosynthesis, possibly due to the lack of a favourable furin-like cleavage site (SLRR↓ST). In this case, it was reported that following receptor binding the S-protein is cleaved at a conserved sequence AYT↓ST by target cells’ proteases such as elastase, cathepsin L or TMPRSS2 (Bosch et al., 2008; Matsuyama et al., 2010, 2005; Millet and Whitaker, 2015). As the priming event is essential for virus entry, the efficacy and extent of this activation step by the proteases of the target cells should regulate cellular tropism and viral pathogenesis. In the case of the 2019-nCoV S-protein, the conserved site 2 sequence AYT↓M may still be cleaved, possibly after the preferred furin-cleavage at the site 1 (Fig. 2).

Since furin is highly expressed in lungs, an enveloped virus that infects the respiratory tract may successfully exploit this convertase to activate its surface glycoprotein (Bassi et al., 2017; Mbikay et al., 1997). Before the emergence of the 2019-nCoV, this important feature was not observed in the lineage b of betacoronaviruses. However, it is shared by other CoV (HCoV-OC43, MERS-CoV, MHV-A59) harbouring furin-like cleavage sites in their S-protein (Fig. 2; Table 1), which were shown to be processed by furin experimentally (Le Coupenc et al.,...
2015; Mille and Whittaker, 2014). Strikingly, the 2019-nCoV S-protein sequence contains 12 additional nucleotides upstream of the single Arg sequence, which corresponds to a canonical furin-like cleavage site (Braun and Sauter, 2019). The 2019-nCoV S-protein is expected to be cleaved at site 2 during virus endocytosis, as observed for the SARS-CoV.

Obviously much more work is needed to demonstrate experimentally our assertion, but the inhibition of such processing enzyme(s) may represent a potential antiviral strategy. Indeed, it was recently shown that in an effort to limit viral infections, host cells that are infected by a number of viruses provoke an interferon response to inhibit the enzymatic activity of furin-like enzymes. It was also demonstrated that HIV infection induces the expression of either the protease activated receptor 1 (PAR1) (Kim et al., 2015) or guanylate binding proteins 2 and 5 (GBP2,5) (Braun and Sauter, 2019) that restrict the trafficking of furin to the trans Golgi network (PAR1) or to early Golgi compartments (GBP2,5) where the proprotein convertase remains inactive. Altogether, these observations suggest that inhibitors 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 harboured α1-antitrypsin variant. J. Biol. Chem. 268, 24887–24891.

References


Decroly, E., Wouters, S., Di Belio, C., Lazure, C., Raemy, S., Van Damme, P., Mille, C., Whittaker, A.D., 2014. Strikingly, the 2019-nCoV S-protein is expected to be cleaved at site 2 during virus endocytosis, as observed for the SARS-CoV.


