SARS-CoV-2 spike and its adaptable furin cleavage site

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Much attention has been drawn to the origin of SARS-CoV-2, the causative agent of COVID-19. One notable feature of SARS-CoV-2 is a four-amino acid insert starting with proline (SPRRAR|S) at the junction of the receptor-binding (S1) and fusion (S2) domains of the spike protein. Following the release of the SARS-CoV-2 genome, several groups identified this insert as a potential cleavage site for the protease furin—the insert has also been referred to as a polybasic site and proposed to be part of the proximal origin of the ongoing pandemic.[1] Proteolytic cleavage is widely used to activate the fusion machinery of viral glycoproteins. However, studies on coronaviruses—including SARS-CoV-2—have shown that activation of the spike proteins of these particular viruses is not straightforward, and is often a complex process involving more than one cleavage event at distinct sites and with the involvement of several host proteases.[2] As such, any consideration of spike protein origin and function need to be considered along with the natural history of coronaviruses and how the spike protein adapts to a milieu of different species, tissue types, and cell types.[3,4]

Furin is ubiquitously expressed in the Golgi apparatus of all cells,[5] but generally only at low levels. Furin has a well known role in viral pathogenesis and efficiently cleaves polybasic or multi-basic sites such as those found in influenza virus subtypes H5 and H7. These highly pathogenic avian influenza viruses have therefore served as a model for the role of furin cleavage as a viral virulence factor. Mechanistically, this furin site is created through polymerase slippage during replication and occurs at the interface of the HA1 and HA2 subdomains. Such polybasic sites typically exist as a stretch of 6–7 arginine and lysine residues (eg, RKKRKR|G) that can be efficiently cleaved by furin, thereby allowing systemic spread based on the ubiquitous expression of the protease.[6] Without the polybasic cleavage site, infection is restricted on the basis of the localised presence of the trypsin-like proteases activating low pathogenicity influenza viruses. Other influenza viruses (such as H9) modulate the cleavage site sequence through mutation and recombination.

For coronaviruses, furin cleavage sites at the interface of the S1 and S2 domain are not unusual, being found widely in betacoronaviruses in the embeco lineage (which are considered to be of rodent origin) as well as in avian-origin gammacoronaviruses and certain feline and canine alphacoronaviruses (with an unknown origin). Furin cleavage sites are also found in certain bat-origin MERS-like merbecovirises, but not—with the exception of SARS-CoV-2—in the sarbecovirus lineage. The presence of a furin cleavage motif at the SARS-CoV-2 S1–S2 interface is therefore highly unusual, leading to the smoking gun hypothesis of manipulation that has recently gained considerable attention as a possible origin of SARS-CoV-2. However, with analogy to influenza, it was shown many years ago that the simple insertion of a polybasic site into an H3 virus does not result in a high pathogenicity phenotype[7] and is likely to only function in the context of a series of other genomic changes provided by a process of natural selection.

So far, a viable natural origin for the SARS-CoV-2 S1–S2 site through recombination or mutation of a bat-origin virus has proved to be elusive. Of note, the S1–S2 cleavage site of SARS-CoV-2 S does not comprise the pattern found in prototypical furin cleavage sites (it is RRxR and not RxK/RR),[8] making its origin enigmatic. One feature of the S1–S2 junction for the SARS-CoV-2 spike from the original outbreak is the presence of a leading proline residue, which might have promoted furin cleavage. As new variants emerged, the leading proline was first replaced by a histidine in B.1.1.7 and now with an arginine in variants such as B.1.617[9,10] to turn the tri-basic PRRAR|S sequence to RRRAR|S, with these cleavage site changes occurring on the background of other genomic adaptations. However, such variant cleavage sites are still not ideal for furin—as would be found in the prototype embecovirus mouse hepatitis virus (RRARR|S)—but do appear to be making S1–S2 more polybasic as the pandemic continues and transmissibility increases. We always need to remember not to oversimplify the complex process of spike protein activation; however, it will be interesting to see whether this progression of basic residue addition continues with new variants, towards that seen in established community-acquired respiratory coronaviruses such as HCoV-HKU-1 or HCoV-OC43—both embecoviruses with S1–S2 sequences of RRKRR|S and RRSRR|A, respectively.

I declare no competing interests.

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