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Structural basis for receptor recognition by the novel coronavirus from Wuhan

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Abstract

A novel SARS-like coronavirus (2019-nCoV) recently emerged from Wuhan, China and is quickly spreading in humans. A key to tackling this epidemic is to understand the virus's receptor recognition mechanism, which regulates its infection, pathogenesis, and host range. 2019-nCoV and SARS-CoV recognize the same host receptor ACE2. Here we determined the crystal structure of 2019-nCoV receptor-binding domain (RBD) (engineered to facilitate crystallization) in complex of human ACE2.

Compared with SARS-CoV, an ACE2-binding ridge in 2019-nCoV RBD takes more compact conformations, causing structural changes at the RBD/ACE2 interface. Adaptive to these structural changes, several mutations in 2019-nCoV RBD enhance ACE2- binding affinity, contributing to the high infectivity of 2019-CoV. These mutations also reveal the molecular mechanisms of the animal-to-human transmission of 2019-nCoV. Alarmingly, a single N439R mutation in 2019-nCoV RBD further enhances its ACE2- binding affinity, indicating possible future evolution of 2019-nCoV in humans. This study sheds light on the epidemiology and evolution of 2019-nCoV, and provides guidance for intervention strategies targeting receptor recognition by 2019-nCoV.

Introduction

The sudden emergence and quick spread of a novel SARS-like coronavirus (2019- nCoV) from Wuhan, China is endangering global health and economy [1-5]. In just two months, 2019-nCoV has caused more infections, deaths, and economic disruptions than did the 2002-2003 SARS coronavirus (SARS-CoV) [6-9]. The origin of 2019-nCoV remains a mystery. Although bats are considered the original source of 2019-nCoV [10-12], the molecular events that led to bat-to-human transmission of 2019-nCoV are not known.

Also unknown is whether 2019-nCoV will evolve further to become even more damaging. Currently there are no clinically proved vaccines or drugs that specifically target 2019-nCoV. Receptor recognition by coronaviruses is one of the most important determinants of viral infectivity, pathogenesis, and host range [13-16]. It is also a major target for vaccination and antiviral strategies [17,18]. Therefore, there is an urgent need to understand the receptor recognition by 2019-nCoV.

Receptor recognition by SARS-CoV has been extensively studied. A virus-surface spike protein mediates coronavirus entry into host cells. SARS-CoV spike protein contains a receptor-binding domain (RBD) that specifically recognizes angiotensin- converting enzyme 2 (ACE2) as its receptor [15,19,20]. We previously determined a series of crystal structures of SARS-CoV RBD (from different SARS-CoV strains) complexed with ACE2 (from different animal species) [15,20-22]. These structures showed that SARS- CoV RBD contains a core structure and a receptor-binding motif (RBM); the RBM makes all of the contacts with ACE2 [20]. The surface of human ACE2 contains two virus- binding hotspots that are critical for SARS-CoV binding. Several naturally selected mutations in SARS-CoV RBM surround these two virus-binding hotspots and regulate the infectivity, pathogenesis, and cross-species and human-to-human transmissions of SARS-CoV [20-23].

Because of the sequence similarity between SARS-CoV and 2019-nCoV spikes, we recently predicted that 2019-nCoV also uses human ACE2 as its receptor [24], consistent with two other studies [10,25]. Here we determined the structural basis for receptor recognition by 2019-CoV. Our findings identify the molecular and structural features of 2019-nCoV RBM that may contribute to the epidemiology of the current 2019-nCoV outbreak. Our study also provides insights into the animal origin and future evolution of 2019-nCoV, and can guide structure-based intervention strategies targeting 2019-nCoV/ACE2 interactions.

Results

To understand the structural basis for human ACE2 (hACE2) recognition by 2019-nCoV, we aimed to crystallize the 2019-nCoV RBD/hACE2 complex. To facilitate crystallization, we engineered 2019-nCoV RBD based on the following rationale. We previously crystallized the SARS-CoV RBD/hACE2 complex [20]. In this crystal form, the core structure of SARS-CoV RBD was mainly involved in crystal lattice contact. Hence, we designed a chimeric RBD containing the core structure from SARS-CoV RBD (as the crystallization scaffold) and the RBM region from 2019-nCoV (as the functionally relevant unit) (Fig. 1A; Fig. S1). To further enhance crystallization, we aimed to improve the binding affinity between the chimeric RBD and hACE2. To this end, we kept in the chimeric RBD a short loop from SARS-CoV RBM to maintain a strong salt bridge between Arg426 from the RBD and Glu329 from hACE2 (Fig. 1B). This loop sits on the side of the binding interface and is away from the main binding interface. We expressed and purified the chimeric RBD and hACE2, and crystallized their complex under the same condition and in the same crystal form as those for the SARS-CoV RBD/hACE2 complex. We then collected the X-ray diffraction data, and determined the structure of the chimeric RBD/hACE2 complex by molecular replacement using the structure of the SARS-CoV RBD/hACE2 complex as the search template. We refined the structure to 2.86 Å (Table S1; Fig. S2).

The overall structure of the chimeric RBD/hACE2 complex is similar to that of the SARS-CoV RBD/hACE2 complex (Fig. 1A). Like SARS-CoV RBM, 2019-nCoV RBM forms a gently concave surface with a ridge on one side; it binds to the exposed outer surface of the claw-like structure of hACE2 (Fig. 1A; 1C). Surprisingly, the strong salt bridge between SARS-CoV RBD and hACE2 became a weaker, but still energetically favorable, N-O bridge between Arg439 from the chimeric RBD and Glu329 from hACE2 [26]. We measured the binding affinities between the three RBDs (2019- nCoV, chimeric, and SARS-CoV) and hACE2 using surface plasmon resonance assay (Fig. 1D; Fig. S3). The result showed that chimeric RBD has higher hACE2-binding affinity than 2019-nCoV RBD, suggesting that our design of a chimeric RBD with enhanced hACE2-binding affinity was successful. Both the chimeric RBD and 2019- nCoV RBD have significantly higher hACE2-binding affinity than SARS-CoV does. We will discuss this further.

A significant structural difference between 2019-nCoV and SARS-CoV RBMs is the conformations of the loops in the ACE2-binding ridge (Fig. 2A, 2B). Compared SARS-CoV RBM, the ridge loops in 2019-nCoV are more compact. An inspection of the RBM sequences reveals that the ridge contains the only insertion in the whole RBM (Fig. 2C). More specifically, SARS-CoV strains from human and civet as well as bat virus Rs3367 all contain a three-residue motif proline-proline-alanine in one of the loops; the tandem prolines

allow the loop to take a sharp turn. In contrast, 2019-nCoV and bat virus RsTG13 both contain a four-residue motif glycine-glutamine/valine- threonine/asparagine-glycine; two relatively bulky residues and two flexible glycines allow the loop to take a different conformation. Because of the above structural differences, an additional main chain hydrogen bond forms between Thr487 and Ala475 in 2019-nCoV RBM, causing the ridge to take a more compact conformation and move closer to hACE2. Consequently, the ridge in 2019-nCoV RBM forms better contact with hACE2. For example, a new hydrogen bond forms between the carbonyl oxygen of Ala475 from 2019-nCoV RBM and the side chain of the N-terminal residue Ser19 from hACE2. Compared with SARS-CoV RBM, these structural changes in 2019-nCoV RBM are more favorable for hACE2 binding.

In addition to the ACE2-binding ridge, compared with the SARS-CoV/hACE2 interface, more structural changes at the 2019-nCoV RBM/hACE2 interface take place (Fig. 3A, 3B). Previously we identified two virus-binding hotspots on hACE2 [21,22]: hotspot Lys31 (i.e., hotspot-31) consists of a salt bridge between Lys31 and Glu35, whereas hotspot Lys353 (i.e., hotspot-353) consists of a salt bridge between Lys353 and Asp38. Both of the hotspots are buried in a hydrophobic environment. Hotspot-31 requires support from a hydrophobic cluster between the hotspot and the ridge, and hotspot-353 requires support from the side chain of Thr487 from SARS-CoV RBM. At the 2019-nCoV RBM/hACE2 interface, because of the structural change in the ridge, several key interactions at the binding interface have rearranged. For example, compared with the corresponding Leu472 in SARS-CoV RBM, Phe486 in 2019-nCoV RBM points to a different direction and forms new contacts at the interface. Because of these structural changes, the hotspot-31 structure has rearranged: the salt bridge between Arg31 and Glu35, both from ACE2, breaks apart, and each of them forms a hydrogen bond with Gln493 from 2019-nCoV RBM. Moreover, due to the presence of Asn501, the salt bridge in the hotspot-353 structure becomes more stable, as evidenced by fact that the salt bridge distance shortens from 3.3 to 2.9 Å. Overall, compared with SARS-CoV RBM, 2019- nCoV RBM is better suited for hACE2 binding.

Discussion

The current 2019-nCoV outbreak has the potential to become a global pandemic. Our previous decade-long structural studies on SARS-CoV have established receptor recognition as one of the most important determinants of SARS-CoV infectivity, pathogenesis, and host range [15,23]. Based on the newly discovered structural basis for receptor recognition by 2019-nCoV presented here, we discuss some of the most pressing questions regarding the virus.

Why is 2019-nCoV more damaging than SARS-CoV? Our study found that compared with SARS-CoV, structural changes in the hACE2-binding ridge in 2019- nCoV RBM cause the ridge to become more compact, get closer to hACE2, and form better contact with hACE2. Moreover, both of the virus-binding hotspots on hACE2 have become more stabilized through interactions with 2019-nCoV RBM. Our biochemical data confirm that 2019-nCoV RBD has significantly higher hACE2-binding affinity than SARS-CoV RBD. Thus, both of the structural and biochemical data reveal that 2019- nCoV recognizes hACE2

better than SARS-CoV does, suggesting more efficient cell infectivity, higher human infectivity, and wider spread of 2019-nCoV.

How did 2019-nCoV transmit from bats to humans? First, like 2019-nCoV, bat RaTG13 RBM contains a similar four-residue motif in the ACE2-binding ridge (Fig. 3C), supporting RaTG13 as the original source for 2019-nCoV. As this study shows, the four- residue motif is correlated with better receptor usage; RaTG13 likely evolved this motif as an adaptation to bat infections (Fig. 4). Second, because residue 329 in bat ACE2 is an asparagine (Fig. 3D), it cannot form a salt bridge with Arg426 from the RBM, allowing this RBM residue to evolve to other residues, first to a lysine in Bat RaTG13 and then to an asparagine in 2019-nCoV. Thus, Asn439 is a viral adaptation to bat ACE2 (Fig. 4).

Third, Gln493 in 2019-nCoV RBM plays a critical role in stabilizing hotspot-31. It is better suited for hACE2 binding than the other residues in the same position in other viral RBMs (Fig. 3C). Hence Gln493 is a viral adaption to hACE2 (Fig. 4). It is worth noting that a lysine-to-asparagine mutation in this same position allowed SARS-CoV to get into human populations [20]. Overall, the combinations of the above critical receptor-binding features of 2019-nCoV may have facilitated 2019-nCoV to transmit from bats to humans, either directly or through an intermediate host (Fig. 4).

Could 2019-nCoV evolve further in the future to become even more dangerous for humans? The chimeric RBD used for crystallization shows that an N439R mutation in 2019-nCoV RBM significantly enhances hACE2 recognition by 2019-nCoV. This mutation may occur as the virus further adapts to human infections, and hence should be closely monitored as an important part of epidemic surveillance.

What does our study inform on intervention strategies? First, neutralizing monoclonal antibodies targeting 2019-nCoV RBM can prevent the virus from binding to ACE2, and hence are promising antiviral drugs. However, the strong binding between 2019-nCoV RBD and human ACE2 suggests that antibody drugs will have a worse chance to outcompete ACE2 for the binding site on the RBD, making this antiviral approach more challenging. Second, the RBD itself can function as a subunit vaccine[17,18]. Our study has identified all of the functionally important epitopes in 2019-nCoV RBM, and hence vaccine design can focus on these epitopes. Moreover, we previously developed a structure-based strategy to improve the efficacy of coronavirus RBD vaccines. This strategy may be helpful in designing 2019-nCoV RBD vaccines [27].

Finally, antibody-dependent enhancement (ADE) of 2019-nCoV infection should be carefully monitored for secondary 2019-nCoV infections, for antibody-based drug therapy, and for vaccinations. The molecular mechanism of ADE that we recently identified for other coronaviruses may apply to 2019-nCoV [28]. Overall, our study can guide structure-based intervention strategies that target receptor recognition by 2019- nCoV.

Materials And Methods

Plasmids

2019-nCoV spike (GenBank accession number MN908947.1), SARS-CoV Spike (GenBank accession number AFR58742), and human ACE2 (GenBank accession number NM_021804) were all synthesized (GenScript Biotech). 2019-nCoV, SARS-CoV, chimeric RBDs (see Fig. S1 for residue ranges of RBDs), and human ACE2 ectodomain (residues 1-615) were subcloned into pFastBac vector (Life Technologies) with a N- terminal honeybee melittin signal peptide and C-terminal His6 tag.

Protein expression and purification

All of the proteins were prepared from sf9 insect cells using the Bac-to-Bac system (Life Technologies) as previously described [22,29,30]. Briefly, the His6-tagged proteins were harvested from cell culture medium, purified on Ni-NTA column, purified further on Superdex200 gel filtration column (GE Healthcare), and stored in a buffer containing 20 mM Tris pH7.2 and 200 mM NaCl.

Crystallization and structure determination

To purify the RBD/ACE2 complex, ACE2 and RBD were incubated together, and then the complex was purified on gel filtration chromatography. RBD/ACE2 crystals were grown in sitting drops at room temperature over wells containing 100 mM Tris (pH 8.5), 18-20% PEG 6000, and 100 mM NaCl. Crystals were soaked briefly in 100 mM

Tris (pH 8.5), 30% PEG 6000, 100 mM NaCl, and 30% ethylene glycol before being flash-frozen in liquid nitrogen. X-ray diffraction data were collected at the Advanced Photon Source beamline 24-ID-E. Data processing was done using HKL2000 [31]. The structure was determined by molecular replacement using the structure of hACE2 complexed with SARS-CoV RBD as the search template (Protein Data Bank accession code 2AJF). Molecular replacement and model refinement were done using PHENIX and CCP4 [32,33]. Model building was done using COOT [34]. Structural figures were made using PYMOL (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.).

Structure data and refinement statistics are shown in Table S1.

Protein-protein binding assay

Surface plasmon resonance assay using a Biacore 2000 system (GE Healthcare) were carried out as described previously [29,35]. Briefly, different RBDs were immobilized to a CM5 sensor chip (GE Healthcare). The running buffer composed of 10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA and 0.05% Tween 20. Serial dilutions of purified recombinant hACE2 were injected ranging in concentration from 5 to 80 nM for

2019- nCoV RBD and chimeric RBD, and 20-320 nM for SARS-CoV RBD. The resulting data were fit to a 1:1 binding model using Biacore Evaluation Software (GE Healthcare).

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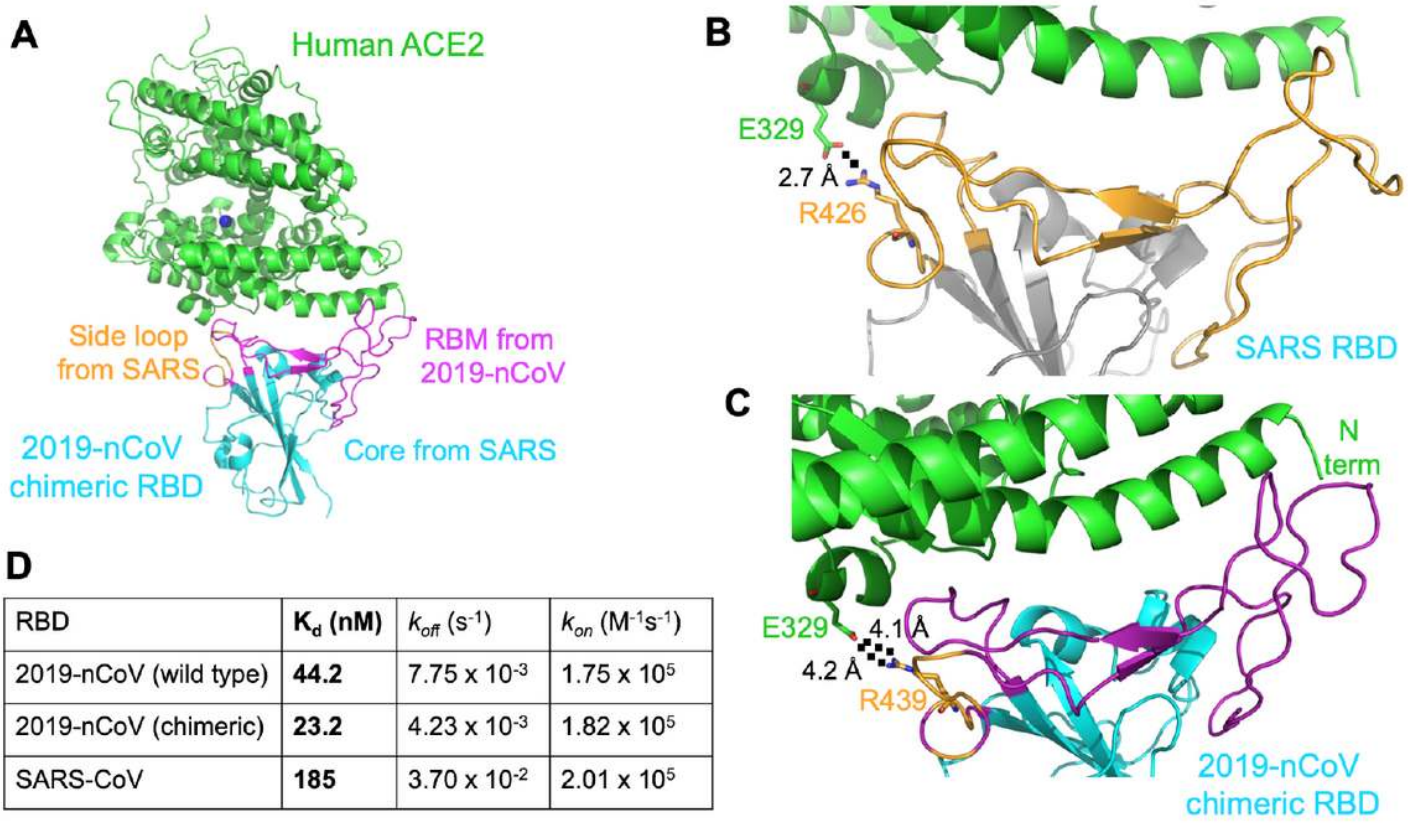
Figures



D

RBD	**K_d (nM)**	k_{off} (s^{-1})	k_{on} ($M^{-1}s^{-1}$)
2019-nCoV (wild type)	**44.2**	7.75×10^{-3}	1.75×10^{5}
2019-nCoV (chimeric)	**23.2**	4.23×10^{-3}	1.82×10^{5}
SARS-CoV	**185**	3.70×10^{-2}	2.01×10^{5}

Figure 1

Overall structure of 2019-nCoV chimeric RBD complexed with human ACE2. (A) Crystal structure of 2019-nCoV chimeric RBD complexed with human ACE2. ACE2 is in green. RBD core structure is in cyan. RBM is in magenta. A side loop in RBM is in orange. A zinc ion in the ACE2 active site is in blue. (B) Interface between SARS-CoV RBM and human ACE2, showing a strong salt bridge between Arg426 on the side loop in the RBM and Glu329 from human ACE2. Core structure is in grey. RBM is in orange. (C) Interface between 2019-nCoV RBM and human ACE2, showing a weaker, but still energetically favorable, N-O bridge between Arg439 on the side loop in the RBM and Glu329 from human ACE2. (D) Binding affinities between the RBDs and human ACE2 as measured using surface plasmon resonance.

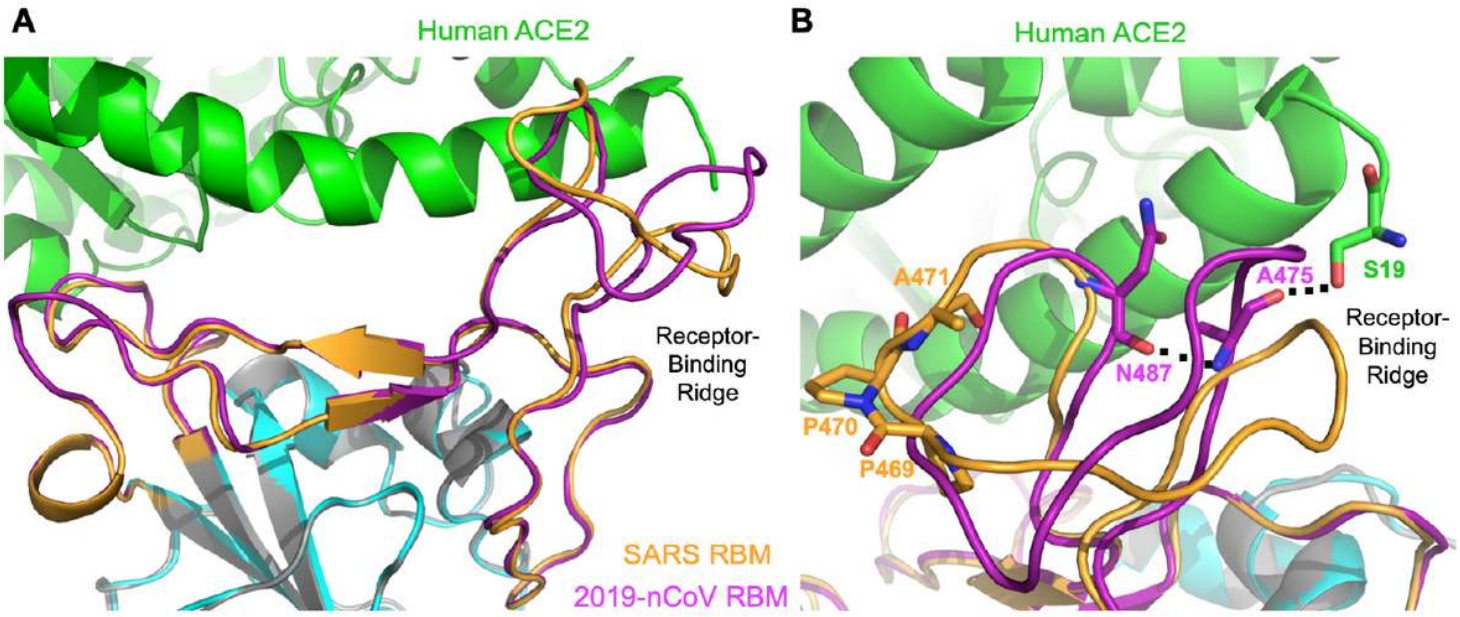
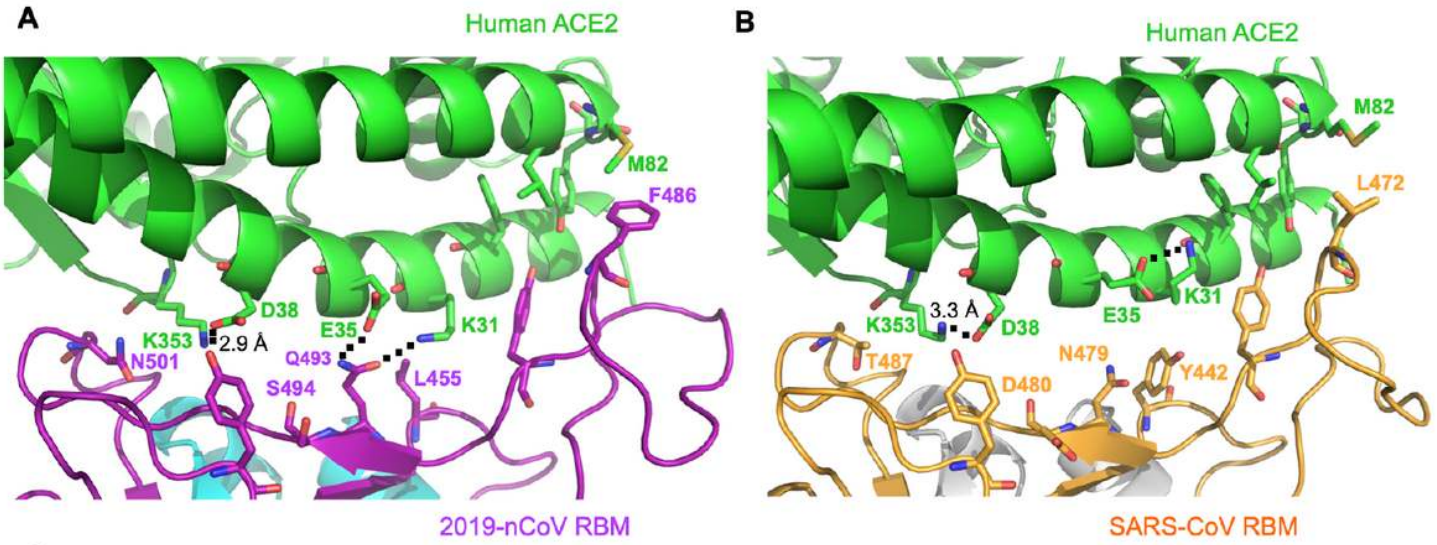


Figure 2

Structural differences in an ACE2-binding ridge between 2019-nCoV and SARS-CoV RBMs. (A) Comparison of the conformations of the ridge in the two RBMs. 2019-nCoV RBM is in purple. SARS-CoV RBM is in orange. (B) Comparison of the conformations of the ridge from another angle of view. A proline-proline-alanine motif in SARS-CoV RBM is shown. Two newly formed hydrogen bonds in 2019-nCoV RBM are also shown. (C) Sequence alignment of RBMs from SARS-CoV and SARS-like viruses. Five previously identified critical ACE2-binding residues are shaded. The significant residue changes in the ACE2-binding ridge are in blue. GenBank accession numbers are: MN908947.1 for 2019-nCoV spike; AFR58742 for SARS-CoV spike; AY304486.1 for civet SARS-CoV spike; MG916901.1 for bat Rs3367 spike; MN996532.1 for bat RaTG13 spike.



C

Viral RBD	Year	426	442	469-471	472	479	480	487
SARS - human	2002	R	Y	P-PA	L	N	D	T
SARS - civet	2002	R	Y	P-PA	L	K	D	S
Bat RS3367	2013	R (427)	S (443)	P-PA (469-472)	F (473)	N (480)	D (481)	N (488)
Bat RaTG13	2020	K (444)	L (464)	GQTG (491-494)	L (495)	Y (502)	R (503)	N (510)
2019-nCoV	2019	N (439)	L (455)	GQTG (482-485)	F (486)	Q (493)	S (494)	N (501)

D

ACE2	19	31	35	38	82	329	353
Human	S	K	E	D	M	E	K
Bat	S	K	K	D	N	N	K

Figure 3

Structural details at the interface between 2019-nCoV RBM and human ACE2. (A) Interface between 2019-nCoV RBM and human ACE2. (B) Interface between SARS-CoV RBM and human ACE2. (C) Comparison of important ACE2-binding residues in the RBM from SARS-CoV and SARS-like viruses. (D) Comparison of important virus-binding residues in human and bat ACE2.

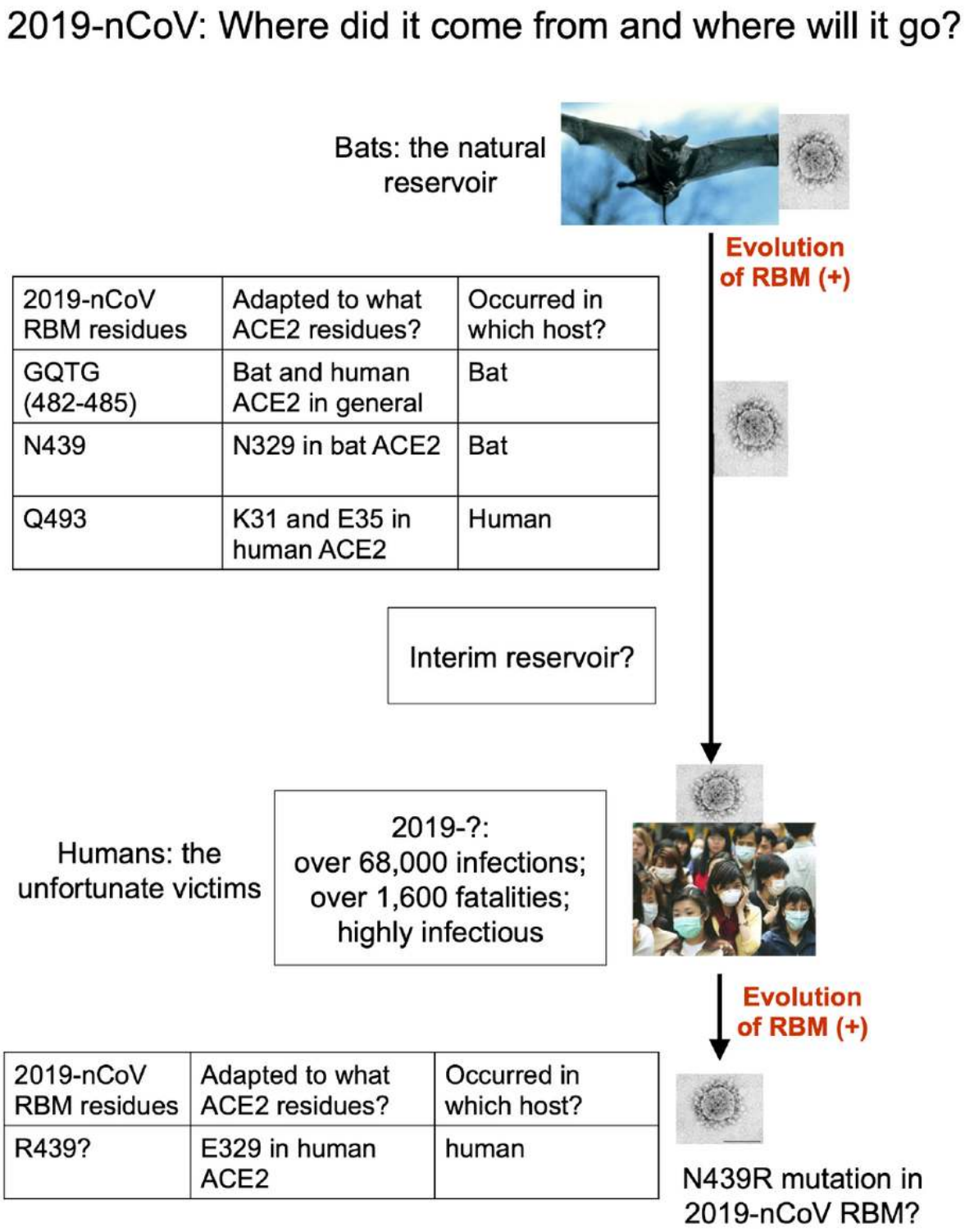


Figure 4

Summary of host adaptation and evolution of 2019-nCoV.

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