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In silico studies on the comparative characterization of the interactions of SARS-CoV-2 spike glycoprotein with ACE-2 receptor homologs and human TLRs

Abhigyan Choudhury | Suprabhat Mukherjee 🄳

Department of Animal Science, Integrative
Biochemistry and Immunology Laboratory,
Kazi Nazrul University, Asansol, West Bengal,
India

Correspondence
Suprabhat Mukherjee, Department of Animal
Science, Kazi Nazrul University, Asansol
713340, India.
Email: babaimbc@gmail.com and
suprabhat.mukherjee@knu.ac.in

Abstract

Coronavirus disease-2019 (COVID-19) outbreak due to novel coronavirus or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has come out as a major threat for mankind in recent times. It is continually taking an enormous toll on mankind by means of increasing number of deaths, associated comorbidities, and socioeconomic loss around the globe. Unavailability of chemotherapeutics/vaccine has posed tremendous challenges to scientists and doctors for developing an urgent therapeutic strategy. In this connection, the present in silico study aims to understand the sequence divergence of spike protein (the major infective protein of SARS-CoV-2), its mode of interaction with the angiotensin-converting enzyme-2 receptor (ACE2) receptor of human and related animal hosts/reservoir. Moreover, the involvement of the human Toll-like receptors (TLRs) against the spike protein has also been demonstrated. Our data indicated that the spike glycoprotein of SARS-CoV-2 is phylogenetically close to bat coronavirus and strongly binds with ACE2 receptor protein from both human and bat origin. We have also found that cell surface TLRs, especially TLR4 is most likely to be involved in recognizing molecular patterns from SARS-CoV-2 to induce inflammatory responses. The present study supported the zoonotic origin of SARS-CoV-2 from a bat and also revealed that TLR4 may have a crucial role in the virus-induced inflammatory consequences associated with COVID-19. Therefore, selective targeting of TLR4-spike protein interaction by designing competitive TLR4-antagonists could pave a new way to treat COVID-19. Finally, this study is expected to improve our understanding on the immunobiology of SARS-CoV-2 and could be useful in adopting spike protein, ACE2, or TLR-guided intervention strategy against COVID-19 shortly.

KEYWORDS
ACE-2 receptor, human TLRs, phylogeny, SARS-CoV-2, spike glycoprotein, therapeutic intervention

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Abhigyan Choudhury and Suprabhat Mukherjee contributed equally to this study.

1 | INTRODUCTION

Novel coronavirus diseases outbreak or coronavirus disease-2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is probably the biggest threat for mankind today. Most of the countries, as many as 225 countries, have become victims of this pandemic viral infection.[1] Hitherto, more than 2.47 lacs death have been recorded alongside 35 lacs individuals are infected with COVID-19 throughout the globe.[1] The first evidence on the recent outbreak of COVID-19 has been diagnosed for causing pneumonia amongst the infected individuals from Wuhan, China at the end of 2019.[2] COVID-19 is considered as a β coronavirus of 2B group and phylogenetic studies revealed that COVID-19 belongs to the family Coronaviridae and order Nidovirales.[3] Alike other flu-causing viruses, COVID-19 is also an RNA containing virus having a protein core/capsid surrounding its RNA genome comprising a positive-sense single-stranded RNA.[4-6] The virus transmits from human-to-human via sneeze, cough, and respiratory droplets.[6,7]

The actual mechanistic insight of the pathogenesis of SARS-CoV2 is yet unclear. This is due to the dearth of knowledge that why the virus has selected human as a principal host and how the virus escapes the human innate immune system. Especially, the interaction between the viral antigens and human Toll-like receptors (TLRs) as well as the mechanistic insights of cytokine storm affecting multiple human organs are particularly unknown. However, the pathophysiology of COVID-19 typically involves entry of the virus through respiratory droplets into the respiratory system, principally the alveoli of lungs, through the airways.[8] The viral glycoprotein on its capsid typically termed as "spike protein" binds to the angiotensin-converting enzyme-2 (ACE-2) receptor and the RNA genome enters the host cell (mostly the alveolar cells) via receptor-mediated endocytosis.[8] After entering the host cell, the viral RNA replicase is formed from the messenger RNA results in rapid replication of the viral RNA and other necessary structural proteins.[9] On the other hand, the interaction between the viral antigen(s) and host immune cells results in the induction of proinflammatory responses which trigger vasodilation, an increase of vascular permeability, and accumulation of humoral factors. All these factors cumulatively cause fever and majorly interrupts gaseous exchange to cause breathing difficulty.[9] Lack of complete understanding about the pathogenesis and immunological peculiarity of the virus made the situation more alarming to mankind. Currently, no specific drug or vaccine is available to challenge this myriad threat. Recent studies have suggested a combination of hydroxychloroquine and azithromycin or drugs used for conventional antiretroviral therapy (remdesivir) could be effective for treating patients with COVID.[10,11] Several studies have attempted to present epitope-based vaccine candidates for the speedy emergence of an effective vaccine against COVID-19.[12,13]

Considering the current scenario of COVID-19 pandemic, the present study aims to add a novel dimension to the existing knowledge of COVID-19 biology. We have presented a comparative picture on the phylogeny, antigenicity, and structural insights on the major infective protein, that is, spike glycoprotein of SARS-CoV-2, SARS-CoV, and Middle East respiratory syndrome-related coronavirus (MERS-CoV) as well as their cognate receptor ACE-2 from different animals through in silico analyses. In addition, we have also explored the involvement of the possible pattern recognition receptors that most likely recognize the viral spike protein and involved in the host-pathogen interaction in SARS-CoV-2 infection in human though biocomputational approach.

2 | MATERIALS AND METHODS

2.1 | Data mining

Full-length amino acid sequences of spike glycoprotein of bat coronavirus RaTG13 (accession number: QHR63300.2), bat SARS-like CoV (accession number: AVP78042.1), bat SARS-like CoV RsSHC014 (accession number: AGZ48806.1), bovine CoV (accession number: CCE89341.1), infectious bronchitis CoV (accession number: QDM39239.1), MERS-CoV (accession number: AUM60024.1), murine hepatitis CoV (accession number: AAW47240.1), palm civet sourced SARS CoV (accession number: AAU04661.1), SARS CoV BJ302 (accession number: AAR07630.1), SARS 2003-related CoV (accession number: ABD72985.1), and SARS-CoV-2 or n-hCoVID-19 (accession number: QIC53213.1) were separately retrieved from the NCBI protein database (www.ncbi.nlm.nih.gov). Similarly, the complete amino acid sequences of transmembrane ACE-2 protein receptors of *Rhinolophus sinicus* (horseshoe bat) (accession number: ADN93475.1), *Manis javanica* (Malayan pangolin) (accession number: XP_017505752.1), and *Homo sapiens* (accession number: AAT45083.1) were also retrieved from the same database.

To study the protein-protein interaction, the 3D structure of the wild-type SARS-CoV-2 spike glycoprotein (PDB ID: 6VYB) was retrieved from the RCSB Protein Data Bank, which was actually determined de novo by using cryo-electron microscopic technique with a resolution of 3.20 Å.[14] Similarly, amino acid sequences of TLR1 (accession number: AAC34137.1), TLR2 (accession number: AAY85650.1), TLR4 (accession number: AAI17423.1), and TLR5 (accession number: AAZ17471.1) were obtained from NCBI protein database (www.ncbi.nlm.nih.gov). 3D structures of each TLR were generated by homology modeling and used in further studies.

2.2 | Phylogenetic analyses of CoV spike (S) glycoprotein and ACE2 receptor

The spike glycoprotein sequences from all the selected organisms were first subjected to multiple sequence alignment using ClustalW algorithm using the MEGA X software suite (Ver. 10.1 64-Bit Windows). Thereafter, the alignments were used for constructing the phylogenetic tree by Neighbor-Joining method in the MEGA platform. Furthermore, phylogenetic reconstruction was performed using bootstrapping analyses with 1000 replications to assess the confidence of the developed phylogenetic tree.

2.3 | Homology modeling and assessment of the stereochemical quality

To study spike protein-ACE interaction, homology modeling was used to model the structure of the ACE2 receptors using the retrieved amino acid sequences using the software MODELLER 9. The stereochemical quality of the so built models was evaluated by plotting Ramachandran Plots using the structural assessment tool provided by the SWISS-MODEL web server (https://swissmodel.expasy.org/).

2.4 | Molecular docking of SARS-CoV-2 spike glycoprotein with ACE-2 receptor and evaluation of the binding affinity

Molecular docking approach was employed to perform a comparative evaluation of the binding affinities of spike protein from SARS-CoV-2 with its cognate ACE2 receptor protein of human as well as other most suspected zoonotic hosts like horseshoe bat and the Malayan pangolin. The study was conducted for each combination of ACE2-spike complex while using shape and electrostatic field correlations and was subjected to optimized potentials for liquid simulations minimization postprocessing. The docking was performed by employing Hex docking software (Ver. 8.0.0) which proves to be a very popular software application for docking molecules even of higher molecular weights. The resultant complex conformations were visualized by through Visual Molecular Dynamics software suite (Ver. 1.9.3).

2.5 | Comparative characterization of the ACE-2 receptor of different animals

The retrieved sequences of the ACE2 receptors of the organisms (horseshoe bat, Malayan pangolin, and human) were used to determine the hydrophobicity based on Kyte-Doolittle algorithm by using ProtScale web server (https://web.expasy.org/protscale/). The server was also employed to construct plots of percent accessible residues for each ACE2 receptor protein. Furthermore, the antigenic propensity of the proteins was also predicted and plotted through Kolaskar and Tongaonkar algorithm. Antigenic peptides finder tool from the immunomedicine group server (http://imed.med.ucm.es). VADAR (volume area dihedral angle reporter) server was also used to assess the structural contents of ACE2 protein.

2.6 | Assessment of relative mutability of MERS, SARS-CoV, and SARS-CoV-2 spike proteins

The relative mutability values for spike proteins of SARS-CoV-2, SARS-CoV, and MERS-CoV were determined from their corresponding amino acid sequences employing ProtScale web server (https://web.expasy.org/protscale/).

3 | RESULTS

3.1 | Analysis of the microevolution of the spike glycoprotein and its receptor

The phylogenetic relationship amongst spike protein of SARS-CoV-2 and its homologs is described in Figure 1A. The phylogenetic reconstruction generated an unrooted phylogenetic tree that resembles a close phylogenetic relation between SARS-CoV-2 and bat CoV RaTG13 and were found to share a monophyletic group (Figure 1A) supported by a high bootstrap value (BV) value of 100. We have also found that spike protein of SARS-CoV does not share the same clade with MERS CoV and, therefore, are phylogenetically distantly related viruses (Figure 1A).

After studying the phylogenetic distance among the major strains of coronavirus, we studied the sequence divergence of ACE2 receptor protein, the major receptor of SARS-CoV-2. Herein, the phylogenetic relationship revealed that ACE2 protein is distinct in character amongst the different genus of the animal kingdom (Figure 1A). For example, human and gorilla are closely related mammals with minimum change in the genome and phylogeny of ACE2 receptor also supports the same Figure 1A. Since this clade is supported by a low BV (50%), it is inferred that the receptor protein in human could have followed a different ancestor than that of the gorilla. Interestingly, ACE2 of the bat was found to be comparatively closer to human ACE2 while pangolin (*M. javanica*) was found to be a distantly related one.

3.2 | Comparative studies on the ACE2 receptor of pangolin, bat, and human

Our phylogenetic analysis revealed a close relationship amongst the spike proteins of SARS-CoV-2 (infecting human) and bat coronavirus. On the other side, the ACE2 receptor found to have distinct characteristics. We have studied the molecular characteristics of ACE2 from human, bat, and pangolin separately (Figure 2). Analyses of the homology model corresponding to pangolin, bat and human ACE2 suggested that both human and bat possess similar content (55%) of alpha-helices in the native structure of ACE2 while pangolin-ACE2 contains 53%. The proportion of beta-sheet in the 3D structures ACE2 of bat, human, and pangolin was 7%, 8%, and 9%, respectively. Relative proportions of random coils in the ACE2 structures were found to be 23%, 35%, and 37%, respectively for ACE2 protein from bat, human, and pangolin origin (Figure 2A).

The assumption of any biochemical or biophysical characteristic from the theoretically designed protein structure is dependent on its stereochemical quality. Herein, the Ramachandran plot of ACE2 for each organism demonstrated that most of the residues are present in the favored region and hence possess good stereochemical stability (Figure 2B). The percentage of accessible residues of human ACE2 was found to be close to bat but different from pangolin (Figure 2C). While considering the plots for percentage accessible residues
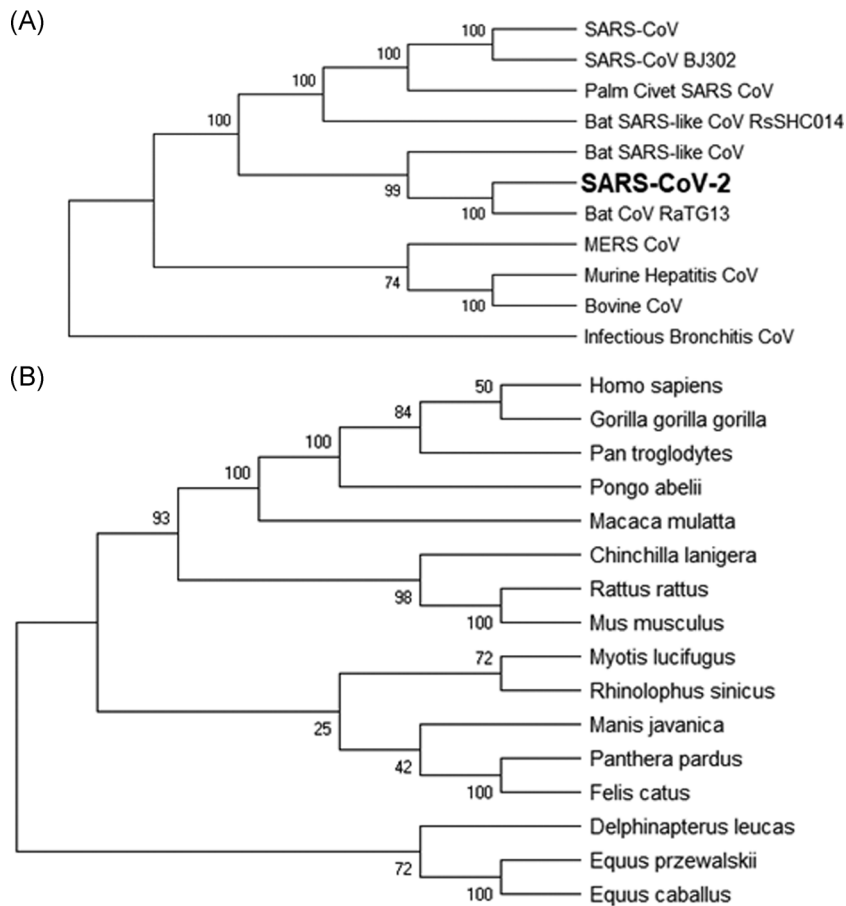
(A)

(B)

FIGURE 1 Phylogeny of spike protein and its cognate receptor ACE2. A, Phylogenetic tree depicting the relationship amongst different coronaviruses. B, Unrooted phylogenetic tree depicting the evolutionary route of human ACE2 receptor. Values indicated in each tree depict the corresponding %BV value. ACE2, angiotensin-converting enzyme-2; BV, bootstrap value

(Figure 2C), it is clear that the ACE2 receptor of *R. sinicus* has much more accessible residues near the ligand-binding region as compared to those of the other two. Hydrophobicity is also considered as an important property of the receptor protein and we have presented the pattern of hydrophobicity of ACE2 from the three different organisms we studied (Figure 2D). The pattern of hydrophobicity of ACE2 of human was also found to follow a similar trend to that of the bat but not to that of the pangolin. In fact, the hydrophobicity plot of bat (*R. sinicus*) showed deeper peaks below the "0.0" midline as compared with the plots of the other two organisms.

3.3 | Assessing the comparative binding efficiency of SARS-CoV-2 spike glycoprotein with ACE-2 receptor

The current debate on the origin of the pathogenic transformation of coronavirus is the origin of the virus which may have a connection with bat or pangolin. To investigate this postulation, we conducted molecular docking of the spike protein of SARS-CoV-2 with the ACE2 receptor of human, bat, and pangolin (Figure 3). We have observed different degrees of binding interactions between spike protein and its receptor (ACE2) from human and two of the most suspected zoonotic hosts/reservoirs (Figure 3). The order of

strength of binding was spike protein-bat ACE2 > spike protein-human ACE2 > spike protein-pangolin ACE2 (Table 1). Interaction between spike protein of SARS-CoV-2 and bat ACE2 was found to be the strongest (Table 1). The interacting surface between spike protein and ACE2 majorly consisting of hydrogen bonding and hydrophobic interaction as depicted in Table 1. Interestingly, the interacting surface of ACE2 of human was also found to be similar to bat Figure 3 and Table 1. Upon close examination, it was observed that the receptor-binding domain of the SARS-CoV-2 spike protein majorly comprises Leu, Glu, Ser, Asn, Phe, Pro, and Asp, while the ligand-binding region of the ACE2 receptors of bat and human are consisted of Glu, Pro, Asn, Ala, and Val (Figure 3 and Table 1). The binding potential of spike protein is also supported by the antigenicity of SARS-CoV-2 that reveals increasing abundance antigenic peptides in SARS-CoV-2 in comparison to the two other strains of coronavirus, that is, SARS-CoV and MERS-CoV (Table S1).

3.4 | TLR-binding efficacy of SARS-CoV-2 spike protein

SARS-CoV-2 pathogenesis principally involves disruption of physio-chemical barriers, while the immunopathological consequences in
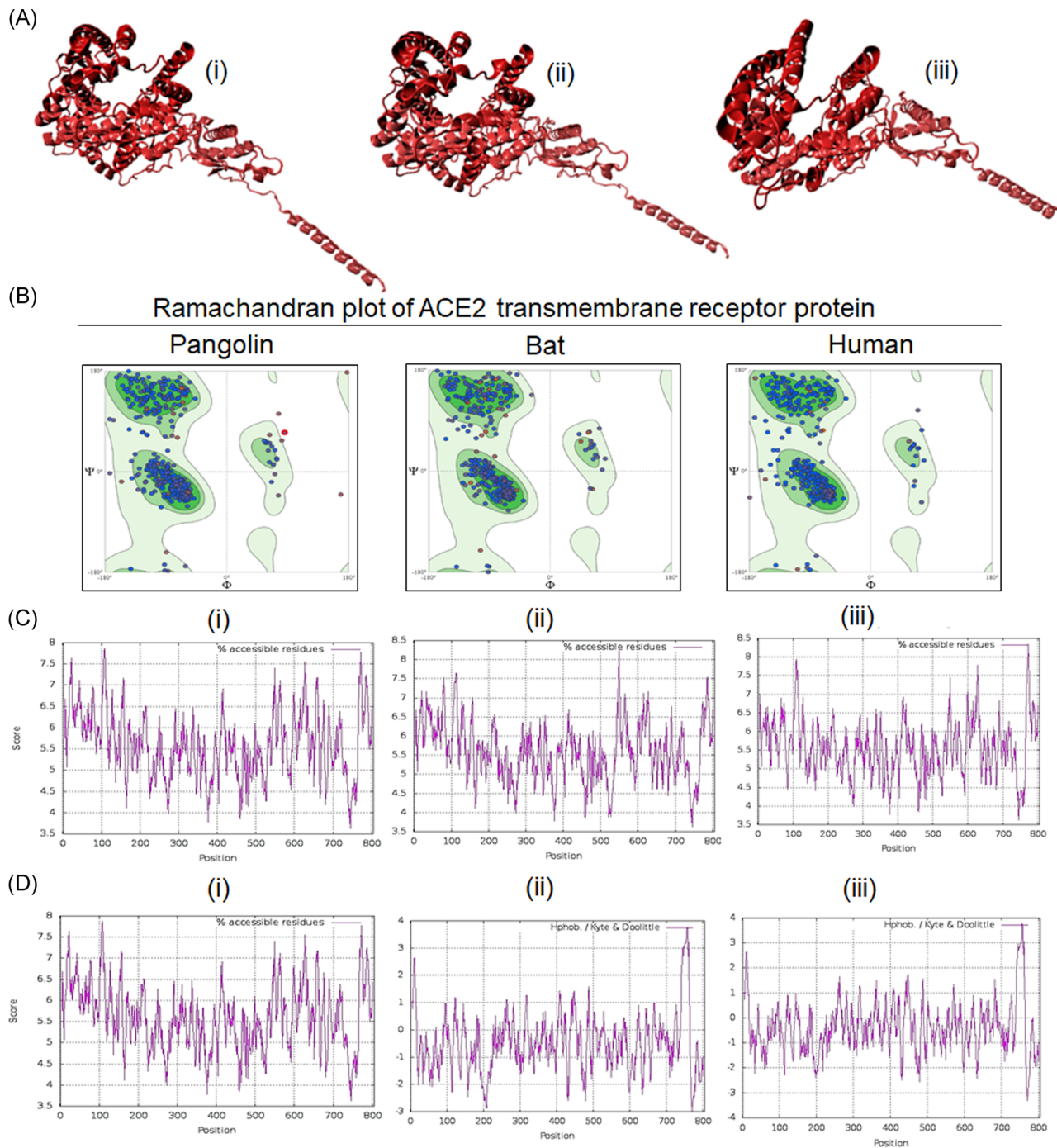
FIGURE 2 In silico characterization of ACE-2 receptor of pangolin, bat, and human. A, Predicted 3D structure of ACE2. B, Ramachandran plot depicting stereochemical quality of the modeled structures of ACE2. C, Accessible residues of ACE2. D, Hydrophobicity in the residues of ACE2. ACE2, angiotensin-converting enzyme-2

human majorly involve respiratory inflammation.[15] Considering this background, we investigated the interaction of the spike protein with the innate immune receptors of human, especially the cell surface TLRs. Molecular docking studies have demonstrated significant binding of the native spike protein of SARS-CoV-2 to TLR1, TLR4, and TLR6 with a respective binding energy value of −57.3, −120.2, and −68.4. Interestingly, TLR4-spike protein interaction has been found to display the strongest protein-protein interaction.

The extracellular domains of the surface TLRs were found to interact with the spike protein in the docked protein complexes (Figure 4). The binding interfaces between the TLRs and the spike protein were consisted of hydrogen bonding and hydrophobic interactions (Table 2). Interestingly, more fine and high valued antigenic peaks found in the antigenicity plot for spike protein were majorly detected at the interacting face of the spike protein, that is, TLR-binding region (Figure S1 and Table S1).
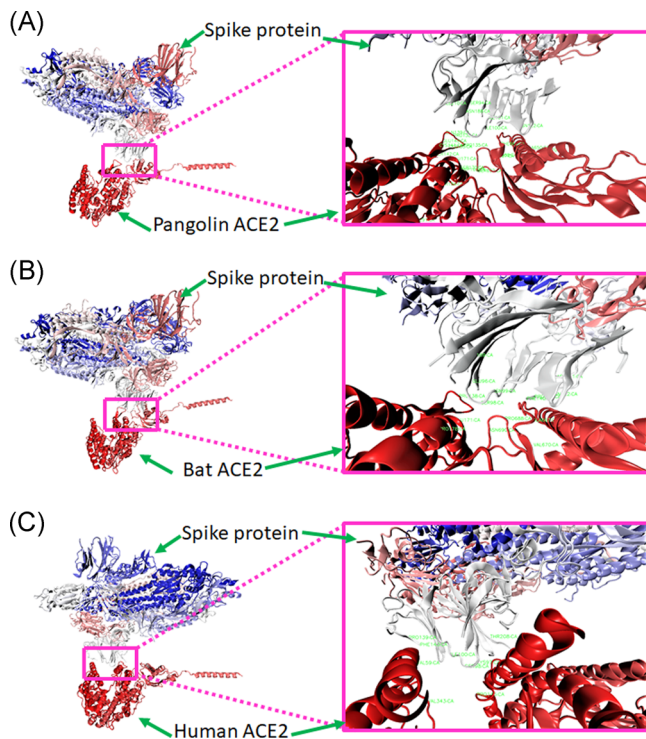
FIGURE 3 Molecular docking showing interaction between the Spike protein of SARS-CoV-2 and its receptor from different host animals. Protein-protein interaction determined through molecular docking showing binding of Spike protein of COVID-19 with ACE2 receptor of (A) pangolin, (B) bat, and (C) human. ACE2, angiotensin-converting enzyme-2; COVID-19, coronavirus disease-2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

3.5 | Analysis of the relative mutability of SARS-CoV-2

In addition to the aforementioned studies, we have analyzed the relative mutability in the ectodomain of the spike protein of three major strains of the coronavirus. We have found that the peptide segment corresponding to S1 subunit of SARS-CoV-2 possesses greater mutability potential as compared with the equivalent peptides present in MERS-CoV and the SARS-CoV. This increased mutability of the spike protein in SARS-CoV-2 most likely to be involved in enhancing its coevolutionary velocity and thus enabling it to conform to various novel microenvironments encountered by it. Moreover, this property may also promote the prevalence of SARS-CoV-2 across wide racial population distributions of human and also letting it penetrate the species-species barrier with greater ease.

4 | DISCUSSION

Coronavirus has established its name in biology through the three pandemic outbreaks, namely, MERS-CoV, SARS-CoV, and the SARS-CoV-2. Out of these, the recent outbreak of COVID-19 pandemic by SARS-CoV-2 has proved to be significantly virulent due its

TABLE 1 Major interacting amino acids involved in spike protein-ACE2 binding interface

Interacting residues		Type of bond	Organism studied	Energy value
Ligand (spike protein) and residue position	Receptor (ACE2)			
Asn122, Asn121, Ser94, Thr208, and His206	Ser113, Gln60, Thr78, Ser124, Tyr127, and Gln139	Hydrogen bond	Human (Homo sapiens)	−29.2
Phe140, Ile210, Leu241, Leu242, Val83, and Pro82	Pro178, Val59, Ala80, and Leu73	Hydrophobic interactions		
Asn137, Ala93, Tyr265, Tyr266, His66, Ser98, Asn121, Asn122, and Thr124	Tyr633, Ser646, Asn636, Tyr641, Cys133, Thr496, and Gln139	Hydrogen bond	Bat (Rhinolophus sinicus)	−44.4
Leu242, Ala93, Phe135, Phe140, and Ile100	Pro178, Met270, Met640, Leu186, Pro138, and Pro688	Hydrophobic interactions		
His66, Ser98, Thr124, Asn211, Asn121, and Asn125	Tyr649, Ser646, Gln175, Thr662, Gln139, and Val670	Hydrogen bond	Pangolin (Manis javanica)	−25.7
Ala123, Ala264, Ile210, Phe65, and Ala27	Pro135, Leu179, Ile126, and Leu642	Hydrophobic interactions		

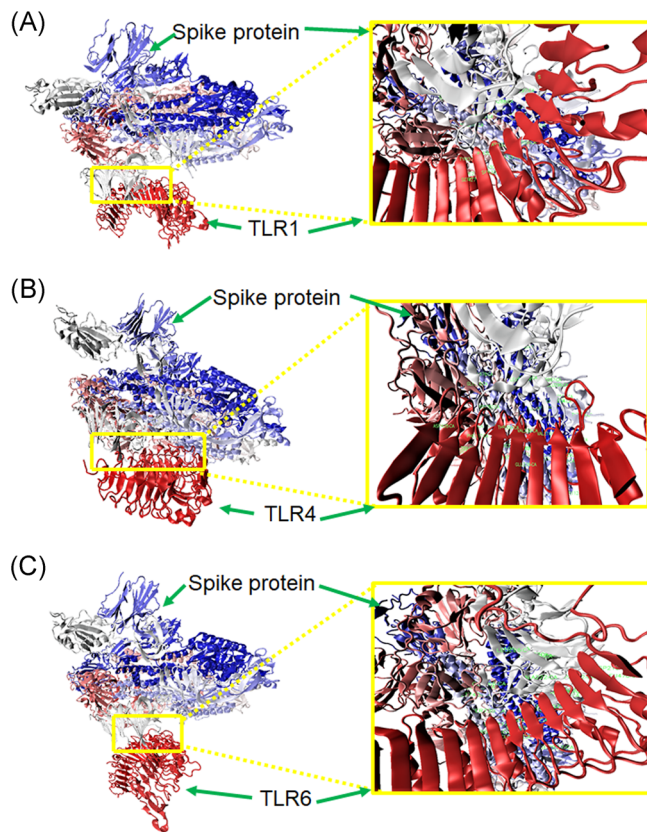Abbreviation: ACE2, angiotensin-converting enzyme-2.

FIGURE 4 Spike protein of SARS-CoV-2 physically interacts with cell surface TLRs of human. Molecular docking showing interaction of spike protein with (A) TLR1, (B) TLR4, and (C) TLR6. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TLR, Toll-like receptor

tremendous ability of infection, transmission, and producing fatal outcomes.[8] There are considerable debates currently going on among the scientific communities regarding the emergence of COVID-19. In this regard, postulations on the emergence of the virus from bat or pangolin, possibilities of emergence of COVID-19 through genetic

modifications, and adaptive mutations and re-emergence of the previously emerged SARS-CoV/MERS, all are open questions to answer. In this context, a recent whole-genome analysis by Zhang et al[16] revealed that Pangolin-CoV and BatCoV (RaTG13) are 90.55% and 91.02% homologous to SARS-CoV-2, respectively. These experimental evidence indicated that pangolin and bat could be the possible origin/source for SARS-CoV-2 and COVID-19 outbreak. Intriguingly, a further report by Andersen et al[17] demonstrated that SARS-CoV-2 is not a laboratory-made or intentionally manipulated virus rather it may have originated through natural selection. Both of these studies have indicated the involvement of zoonotic reservoir.

In the present study, our data suggest that spike protein of SARS-CoV-2 is phylogenetically closer to bat SARS-CoV than that of pangolin SARS-CoV but distantly related to MERS (as described in Figure 1). Previous studies in this direction indicated that SARS-CoV-2 genome (RNA) shares 96% homology with bat coronavirus and 79.5% with SARS-CoV.[18] The present study is corroborated with this and indicated a close relationship between bat SARS-CoV and human SARS-CoV-2. A higher BV of >70% indicated a well-established monophyletic group.[19,20] Moreover, the spike protein of SARS-CoV-2 contains more antigenic peptides than that of the SARS-CoV and MERS (Supplementary Table 1). The present study is, therefore, suggesting that spike protein expression in the virome of SARS-CoV-2 is closely related to that of the Bat CoV RaTG13 and both forms of the virus might have originated from a common ancestor. Moreover, our data also hypothesized that human SARS-CoV-2 is a naturally modified version of bat SARS-Cov. Interestingly, we have also observed that the cognate receptor of spike protein, that is, ACE2 is phylogenetically close between human and bat. This inference was also verified through determining the relative structural content and binding efficacy of the spike protein to human, bat, and pangolin ACE2 receptor (Figures 2 and 3). The structural contents, hydrophobicity and proportion of accessible residues of ACE2 were very close in bat and human while the molecular docking studies revealed that spike protein of SARS-CoV-2 binds more strongly to bat and human than pangolin. Moreover, the interaction zone in spike

TABLE 2 Major interacting amino acids involved in spike protein-TLR-binding interface

Interacting residues				
Ligand (spike protein)	TLR studied	Residue(s) from TLR	Type of bond	Energy value
ASN87, THR51, TYR204, SER305, THR761, THR240, and ASN30	TLR1	SER399, HIS370, THR372, CYS368, and GLN402	Hydrogen bond	−57.3
ILE105, VAL36, PHE58, PHE220, and LEU117		MET397, ALA391, PHE350, and LEU377	Hydrophobic interactions	
SER221, ASN280, THR588, THR208, ASN657, and TYR204	TLR4	ASN409, ASN333, SER386, SER352, HIS431, and ASN361	Hydrogen bond	−120.2
PHE562, LEU226, PRO289, and ILE584		LEU385, VAL411, PHE342, and PHE408,	Hydrophobic interactions	
ASN536, THR581, GLN563, SER221, GLN564, and TYR38	TLR6	HIS350, ASN423, ASN438, ASN387, HIS345, and THR302	Hydrogen bond	−68.4
PHE559, LEU582, LEU552, PHE565, PRO561, and ILE587		PRO349, LEU382, MET335, ILE338, LEU304, and ILE430	Hydrophobic interactions	

Abbreviation: TLR, Toll-like receptor.

protein-ACE2 complex of both bat and human was also found comprising similar residues. This could be the possible reason behind choosing human as a host for SARS-CoV-2. Moreover, an abundance of human than that of bat might have promoted the pathogenic transformation to select human for speedy transmission. We, therefore, studied the involvement of the human innate immune receptors, that is, the TLRs to understand the rationale of choosing the human as a host by SARS-CoV-2 from an immunological point of view.

Immunopathology of COVID infection has been reported to be caused due to the elevated inflammatory response that constitutes an abundant expression of proinflammatory cytokines like interleukin-6 and tumor necrosis factor-alpha.[9] These two cytokines are the products of the TLR signaling pathway[21] and, therefore, we have investigated the role of TLRs in this study. Since, SARS-CoV-2 is an RNA virus, one might ask about the functional role of the TLR-3, 7, 8, and 9. But, the immunopathological outcomes are most likely induced at the point of host-virus interaction, that is, interaction between spike protein of SARS-CoV-2 and immune cells present in the alveoli of human.[8] Therefore, we examined the possible involvement of the surface TLRs present in the human cells and their interaction with the spike protein of SARS-CoV-2. Our data indicated that TLR4 possesses a strong binding affinity to spike protein following TLR6 and TLR1 (Figure 4 and Table 2). TLR4 being the most efficient innate immune receptor that induces proinflammatory responses after binding with the pathogenic ligand.[22] Therefore, the interaction between TLR4 and spike protein could be one the reasons behind the immunopathological manifestation of COVID-19. This interaction could be useful for developing drugs against the same or using TLR4 antagonists as anticorona therapeutics. Since we have used the native conformations of spike protein and TLRs, we have computed the most likely interactions, however, actual protein-protein interaction study using surface plasmon resonance can reveal the actual strength of binding and the binding constant. Intriguingly, we have seen positive energy values for TLR 3, 5, and 6 which indicate a complex interaction that needs to be examined in future. However, a recent study by Bhattacharya et al[12] revealed a vaccine candidate from SARS-CoV-2 spike protein that interacts with TLR5. Since the vaccine is a peptide with much shorter than the actual protein in length and of helical structure, it is much more exposed to the TLR than that of the whole protein complex in its native configuration aimed in this study. Finally, our data on mutability indicated the strongest mutability of SARS-CoV-2 than that of SARS-CoV and MERS-CoV. All these evidence collectively support the earlier studies[16,17,23] that have indicated the origin of SARS-CoV-2 from animals and further strengthen the view on the origin of this viral strain from bat. However, more experimental validations are particularly needed to establish these inferences.

5 | CONCLUSION

The present in silico studies indicated a close relationship between bat SARS-CoV and SARS-CoV-2 at the level of phylogeny of the major infective protein, that is, spike protein and its receptor ACE2 as well as protein-protein interaction between these two. Zoonotic origin of SARS-CoV-2 from bat is, therefore, supported in this study. Spike protein of SARS-CoV-2 has been found to bind with surface TLRs (TLR1, 4, and 6), especially strongly with TLR4. Therefore, selective targeting of TLR4-spike protein interaction by designing competitive TLR4-antagonists could pave a new way to treat COVID-19. Taken together, this study is expected to improve our understanding of the biology of SARS-CoV-2 and the findings contribute to the fundamental knowledge which could be useful in adopting an accurate intervention strategy in near future.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

AC: performed all the experiments, analyzed the data, and assisted in manuscript writing; SM: designed the study, analyzed the data, wrote the manuscript, and supervised the study.

ORCID

Suprabhat Mukherjee https://orcid.org/0000-0002-5709-9190

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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