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Title: Covid19: Probing the possibility of transmission in animal kingdom

Author names and affiliations: Tawsif Ahmed Kazi[1], Suranjita Mitra[1], Bidhan Chandra Mukhopadhyay[1],Rajarshi Bhattacharaya[1], Sukhendu Mandal[2], Swadesh Ranjan Biswas[1*]

[1]Department of Botany, Visva-Bharati, India

[2]Department of Microbiology, University of Calcutta, India

***Corresponding author**: Swadesh Ranjan Biswas, Professor, Department of Botany, Visva-Bharati, India,swadesh_s2000@yahoo.co.in

Abstract

The recent 2019-nCoV outbreak, spreading infection around the globe is jeopardizing the public healthand global economy. The virus was reported to have emerged from an animal market in Wuhan, China at the endof 2019 and presumed to have originated from bats and eventually transmitted in humans. The entry of the virusinto human cells is triggered by a series of molecular events initiated with the binding of a receptor-binding domainof viral spike protein to human Ace2 cell surface receptor. Based on the comparative sequence analysis of thewell-known binding hotspots of human Ace2, cross-interacting potential of 2019-nCoV was predicted, whichsuggests Ace2 of wild animals like tiger, bear, orangutan, etc.; aquatic mammals like whale and dolphins; anddomestic animals like cat, horse, goat, sheep, dog etc. as potential target. However, the recognition of Ace2 ofbats, rats and mice by the 2019-nCoV spike protein remains under question. The study indicates that 2019-nCoVmight have broad host range and may thus intensify the gravity of 2019-nCoV outbreak.

Keywords: 2019-nCoV, Intermediate host, Ace2 receptor, COVID-19

Introduction

In late December 2019, Covid-19, the disease caused by 2019-nCoV reported from animal market inWuhan, China, has now spread almost all over the globe with disastrous effect on humans(Wang et al. 2020).The World Health Organization declared worldwide COVID-19 pandemic for this biggest ongoing issue.Confirmed cases of Covid-19 has spiked to 3,086,832 cases globally, with 212,666 fatality (https://www.worldometers.info/coronavirus/). The animal source of this disastrous outbreak still remains aquestion. It is thus imperative to understand which animals may be susceptible to this virus so as to take precautionary measures to minimize the spread of COVID-19 (Lam et al. 2020; Xia 2020). Like many other positive-sense single-stranded RNA coronaviruses, 2019-nCOV surface spike glycoprotein is an essential weapon, allowing the virus to get into host cells following a specific interaction to the Ace2 receptor of the host cells through the receptor-binding domain (RBD) in the spike (Wang et al.2020). Several amino acid residues (Gln493 and Asn501) of the 2019-nCoV RBD have been identified as critical for effective interaction and entry of virus into the host (Shang et al. 2020; Wan et al. 2020). It is reported that two virus-binding hotspots consisting of a Lys31-Glu35 (hotspot-31) and a Lys353-Asp38 (hotspot-353) salt bridges on human Ace2 are critical for virus-receptor interactions. Moreover, steric hindrance by Asn82, identified in Ace2 receptor of some animals, is known to prevent virus-receptor interaction thereby infection (Fung and Liu 2019). The sequence analysis of the critical residues in the hotspots of Ace2 of different animals and prediction of the virus-receptor interaction, critical for disease development in animals, is the major focus of this study with an objective to envisage the possibility of those animal as a host or target of 2019-nCoV.

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Materials and Methods

Amino acid sequences of Ace2 of different animals were retrieved from NCBInr and UniProt database. Multiple Sequence alignments were performed using MUSCLE v3.8.31 and Clustal Omega (https://www.ebi.ac.uk/Tools/msa/). Phylogenetic analysis of Ace2 was done using MegaX software package(Kumar et al. 2018).

Result and Discussion

Based on the existing knowledge of the molecular and biochemical nature of the interaction between 2019-nCoV spike with human Ace2 receptor, the potential of the 2019-nCoV to recognize Ace2 receptor of several domestic and wild animals has been predicted (Table-1). A comparison of hot spot architecture of Ace2 (Fig.1) of human, suggest rhesus macaque, chimpanzee, gorilla, and orangutans to be potential target for 2019-nCoV. Rats can evade the infectious 2019-nCoV, whereas mouse may be apparently infected inefficiently by 2019-nCoV. Cattles, goat, sheep, and pigshare identical hotspot-31 and 353 with humans Ace2, thus allowing them to effectively interact with 2019-nCoV. Likewise, domestic dogs, cats, tiger, cheetah, horse and red fox, similar to human-Ace2 in hotspot architecture, might be targets for interaction by the virus (Table-1). This interpretation is further supported by the fact that tigers have tested positive for 2019-nCoV at Bronx Zoo, USA (https://www.usatoday.com/). The Ace2 receptor of masked palm civet was also found to interact with 2019-nCoV. Comparative analysis of critical residues of different Ace2-receptors from little brown bat and chinese rufous horseshoe bat revealed that little brown bats might or might not get infected, whereas chinese rufous horseshoe bats are inefficient in interacting with 2019-nCoV. Ace2 of grizzly bear, polar bear, giant panda and aquatic mammals like sperm whale and dolphin was found to be effective for interaction with 2019-nCoV (Fig.1). The viral interaction with the Ace2 of snakes, frogs and chickens is still unclear. In a phylogenetic tree of Ace2 (Fig.1), we observed that 2019-nCoV can interact with various organisms irrespective of the irrelatedness to humans. It is thus suggested that spike protein of 2019-nCoV has the ability to interact with Ace2-receptor of various animals. The study highlighting the virus-receptor interaction in different animals, might be an indicator to predict the potential threats by 2019-nCoV for infection, transmission, and spread in other animals and humans, which might in turn create greater loss for the civilization as a whole. Apart from being a potential threat,identification of animal hosts susceptible to Covid-19 disease, showing symptoms that mimics humans, is also important to build an animal model for drug development research. Frequent monitoring of both terrestrial and aquatic life for isolation and identification of new corona viruses is suggested to check animal trafficking in order to minimize the chances of future outbreaks. Essential investigations on detailed structure-function relationship of these candidate proteins might unravel the truth further.

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Table-1: Comparative analysis of ACE2-receptor hotspots of various organisms with human ACE2 receptor, for successful interaction with 2019-nCOV spike glycoprotein. N-linked Glycosylation sites were identified from UniProt database.

Ace2 Receptor (Accession No./Organism)	**Interaction**	**Reason (Based on the critical residues of hotspots of Ace2 receptor as shown in Fig.1)**
G3QWX4, Gorilla *(Gorilla gorilla);* **A0A2J8KU96, *Chimpanzee*** *(Pan troglodytes);* **F7AH40,** Rhesus macaque *(Macaca mulatta);* **Q9BYF1,** Human *(Homo sapiens);* ***Q5RFN1,*** Orangotan *(Pongo abelii)*	**++**	Hotspot configuration of Ace2 receptor, K31, E35, D38, M82, K353 are identical to that of humans, M82 will not undergo N- linked glycosylation, hence facilitating the interaction with 2019- nCoV.
Q56NL1, Masked palm civet *(Paguma larvata)*	**+**	As compared to human-Ace2, Thr31 of civet-Ace2 will not form a salt bridge with Glu35; E38 of civet-Ace2 favours the formation of a strong bifurcated saltbridge with K353, hence, compatible for2019-nCoV interaction. (Fung and Liu 2019; Wan et al.2020).
E2DHI7, Horseshoe bat *(Rhinolophus sinicus)*	**-**	Glu31 and Glu35 will prevent salt bridge formation at hotspot-31; Asn38 will also prevent salt bridge formation at hotspot-353; N- linked glycosylation at Asn82 will sterically hinder the virus- receptor interaction (Fung and Liu 2019).
E2DHI4, Horseshoe bat *(Rhinolophus sinicus)*	**-**	E35K mutation in the bat-Ace2 will disrupt the hotspot-31; Asn at position 82 will result in N-linked glycosylation (Fung and Liu 2019).
G1PXH7, Little brown bat *(Myotis lucifugus)*	**-**	Salt bridge formation in hotspot-31 is inhibited by K31N, E35K mutation, which in turn will not favour the effective interaction of 2019-nCoV and ACE2 of Little brown bats (Wan et al. 2020)
XP_007090142.1, Tiger *(Panthera tigrisaltaica);* **Q56H28,** Cat *(Felis catus);* **XP_026910297.1,** Cheetah *(Acinonyx jubatus);* **F6V9L3,** Horse *(Equus caballus);* **J9P7Y2,** Domestic Dog *(Canis lupus familiaris);* ***A0A3Q7RAT9,*** *Red_fox (Vulpes vulpes)*	**++**	Salt bridge formation at hotspot-31 and hotspot-353 is favored as that found in human-Ace2. E38 will allow the salt bridge formation in the receptor hotspot-353; Thr82 devoid of N-linked glycan, hence promoting virus-receptor interaction (Fung and Liu2019; Wan et al. 2020).
I3M887,Squirrel *(Ictidomys tridecemlineatus);* **G1TEF4,** European_rabbit *(Oryctolagus cuniculus)*	**++**	Hotspot configuration is similar to that of human-Ace2. T82 (European rabbit) and A82 (Squirrel) devoid of N-linked Glycan, favouring the virus-receptor interaction (Wan et al. 2020).
A0A384CIJ9, Polar bear *(Ursus maritimus);* **A0A3Q7TE16,** Grizzly bear *(Ursus arctoshorribilis);* **G1MC42,**Giant panda *(Ailuropoda melanoleuca)*	**++**	Critical amino acid residues in the hotspots will allow salt bridge formation; lack of N-linked glycosylation will favour the interaction with the spike protein of 2019-nCoV
A0A2Y9S5T9, Sperm whale *(Physeter macrocephalus);* **A0A2U4AJL3,** Dolphin *(Tursiops truncatus)*	**++**	K31, E35, D38, T82, K353, at the virus-receptor interface, similar to human-Ace2 hotspots will favour the receptor-virus interaction.
A0A220QT48, Pig *(Sus scrofa domesticus);* **A0A452EVJ5, Goat***(Capra hircus);* ***Q58DD0,*** Cattles *(Bos taurus);* **W5PSB6,** Sheep *(Ovis aries)*	**++**	Shares identical hotspot-31andhotspot-353, compared to humans; T82 prevents N-linked Glycosylation. Hence facilitating interaction.
Q8R0I0, Mouse *(Mus musculus)*	**-**	Mouse-Ace2 receptor contains His353, disrupting the hotspot- 353; hence, preventing the virus-receptor interaction (Fung and Liu 2019).
Q5EGZ1, Rat (*Rattus norvegicus)*	**-**	His353 disrupts hotspot-353; Asn82 introduces an N-linked glycan (Fung and Liu2019)

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QEQ50331.1, Chicken *(Gallus gallus);* **XP_018104311.1,** Frog *(Xenopus laevis);* **XP_029140508.1,**Snake *(Protobothrops mucrosquamatus)*	**?**	Altered residues at hotspots, as compared to human-Ace2; hence interaction could not be predicted

++ denotes successful interaction; - denotes no interaction; + denotes inefficient interaction; ? means could not be predicted

Fig.1:
The evolutionary history of the Ace2 receptor was inferred using the Neighbor-Joining method. The robustness of the trees, were assessed using 1000 bootstrap replication. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. This analysis involved 31 amino acid sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 882 positions in the final dataset. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The key residues of the hotspots are highlighted in Red, the probable glycosylation site is highlighted in Green. The organisms noncompatible to interact with 2019-nCoV are enclosed in Red box and the interaction could not be predicted for the organisms enclosed in Blue box.