Original Article

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Role of Furin Activation Sites as Receptors for Invasion of Severe Acute Respiratory Syndrome Coronavirus-2 Into Human Cells

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Abstract:

Objective: The severe acute type of respiratory distress caused by Coronavirus disease 2019 (COVID-19) was responsible for the global pandemic of 2019. While most of the focus of vaccine/drug molecules is on the receptor, there are certain enzymes that also need to be checked. Cell surface proteases are one of these. Activation of the virus spike protein becomes more complicated when many host proteases are involved. As many Variants of Concerns have been reported in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), this study aimed to understand the proteolytic function of Furin in each, and its involvement in virus-host interaction.

Material and Methods: Spike Protein sequence alignment, furin cleavage site prediction of variants: Wuhan, Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and Omicron (B.1.1.529), and protein-protein docking studies have been undertaken using appropriate bioinformatics tools.

Results: It was observed that when compared to previous variations, the November 2021, outbreak of Omicron variant showed 50 amino acid substitutions in the Spike protein. Thus, in addition to the Angiotensin Converting Enzyme 2 (ACE-2) receptor, the role of virus binding sites to act as "Addition Receptors" for viral entry has been reported here.

Conclusion: It was observed that substitution of basic amino acids in the Omicron variant may be responsible for the recognition of furin cleavage sites and the presence of furin cleavage site in the receptor binding domain (RBD) region

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will thus enhance viral transmission. If these sites are utilized in formulation of new drugs/vaccine molecules to target the furin hydrolyse sites, we may be able to add to the existing course of COVID-19 treatment.

Keywords: COVID-19, furin, SARS-CoV-2, variants of concern

Introduction

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has appeared as one of the worst pandemics in the recent past. The successful entry, replication, multiplication and exit mechanisms are possible when different viral and host factors interact sequentially throughout the entire cycle. Mechanisms of internalization for the virus into human epithelial cells in the nasopharyngeal and pulmonary regions occur through Angiotensin Converting Enzyme 2 (ACE-2); a surface protein present on the human cells. The precise mechanism in the process of viral entry into human cells involves cleavage of the S1 unit of viral Spike Proteins (SP) by ACE-2, and the S2 unit of S protein helps in attachment of virion with the host cell. In addition to the principle role of viral entry played by ACE-2, higher expressions of TMPRSS2 also facilitates virus internalization[1-6]. Studies conducted in the past on Infectious Bronchitis Viruses (IBVs) suggested that Furin can recognize the S protein of the Beaudette strain of IBVs, and so leads to enhanced infection[7-10]. Furin may thus be considered to be one of the risk factors in the mortality of SARS-CoV-2 infected patients. The alleles rs6224 T and rs4702 A expression are associated with the high risk of mortality in COVID-19 patients[11].

As many Variants of Concerns have been reported in SARS-COV-2, it is very important to understand whether these have impacted the structure of proteases further leading to modification in their functionality. In this present paper, we report the role of yet another receptor appearing as pronounced in Omicron variants: the Furin receptor site on human cells. Since the endoprotease Furin is expressed in many organs, such as the liver, kidney, lungs and small intestine, the virus could additionally or parallel enter through Furin Activation Sites (FAS) in various key organs of the human body[12]. It has been noted that the Furin cleavage site was not present in the earlier forms of Coronaviruses[13] and has emerged widely in the beta coronaviruses lineage. Some Furin cleavage sites were also found in the MERS-CoV, which originated from bats[14-15]. Studies on ACE-2 and TMPRSS2 have been conducted; however, not much work has been performed on Furin. The present paper reports the role of Furin as a viral receptor facilitating the invasion of SARS-CoV-2 into different organs, especially during the course of infection by the Omicron variant.

Material and Methods

Protein sequence alignment

The amino acid sequence in the FASTA format of the Spike Protein of Wuhan (PDB ID: 6VXX), Alpha (PDB ID: 7LWV), Beta (PDB ID: 7VX1), Gamma (PDB ID: 7V79), Delta (PDB ID: 7T9J) and Omicron (PDB ID: 7T9J) variants were procured from the Protein Data Bank Research Collaboratory for Structural Bioinformatics (PDB RCSB). Clustal Omega Sequence Alignment Tool was used to align the sequences and observe for any kind of mutations. Furin Cleavage Site Prediction.

Furin cleavage site prediction

The ProP 1.0 is an online service that uses an ensemble of neural networks to predict Arginine and Lysine pro-peptide sites in Eukaryotic protein sequences. From

this, the prediction of Furin sites can also be checked. The sequence of the Spike Protein of Wuhan, Alpha, Beta, Gamma, Delta and Omicron strains in the FASTA format was submitted and the amino acid sites which were able to cleave with Furin were identified.

Table 1 Cleavage sites of furin in variants of concern for SARS-CoV-2

Variant of Concern	Position	Sequence	Score
Wuhan	685	NSPRRARISV	0.620
Alpha	76	GTNGTKRIFD	0.298
Beta	78	GTNGTKRIFA	0.402
Gamma	815	PSKPSKRISF	0.333
Delta	813	PSKPSKRISF	0.333
Omicron	495	LRSYSFRIPT	0.237

Protein-protein docking

ClusPro, the online protein-protein docking server was used for the docking of the Spike proteins of Omicron and Wuhan variants of the SARS-CoV-2, with the Furin residues of the host. The docking results were further analyzed using the PyMol visualization tool.

Results

Observation of sites of Furin cleavage on the Variants of Concerns:

The amino acid sequence present in the site where Furin cleaves the spike protein was observed. It was seen that there are changes in the sequence of amino acids of Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and Omicron (B.1.1.529) strains with that of the Wuhan strain (Figure 1, Table 1). In the Alpha variant,

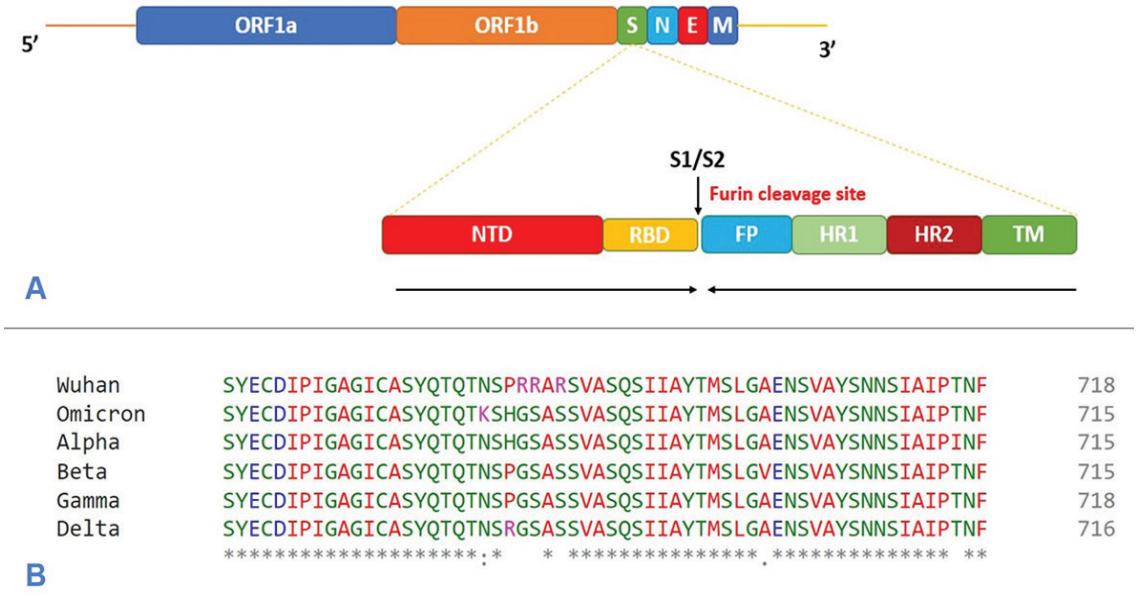


Figure 1 (A) Schematic representation of SARS-CoV-2 Genome. (B) Alignment of Wuhan, Alpha, Beta, Gamma, Delta and Omicron Spike protein at Furin cleavage Site

the site of Furin cleavage disappeared and it was transferred to amino acid position 76, which was not present in the receptor binding domain (RBD) area. When compared with the Wuhan strain of SARS-CoV-2, the Furin Cleavage Site was substituted with the N678K, P680H, R681G, R682S and R684S in the Omicron strain.

Furin proteolysis in S1/S2 site (Spike protein) of the SARS-CoV-2 Wuhan variant

The Prp P1.0 observed a high cleavage potential at two areas (Figure 2). One within the range of 600 to 800, which is the region of Furin interaction, the basic amino acid sequence between the site 678th to 687th was 678 NSPRRARSV 687 (Figures 3 and 4). The second site was seen at positions 76th and 78th; wherein, Threonine and Arginine residues were found (Figure 4).

Furin proteolysis in S1/S2 site (Spike protein) of the SARS-CoV-2 Omicron variant

The spike protein of the Omicron strain of SARS-CoV-2 had mutations, which led to the insertion of basic amino acid sequence, i.e., Lys437, Ser 443, Asn 474, Ser 493, and His 502 compared to earlier strains (Figure 5). The Prop P1.0 software showed a high cleavage potential within one range of 400 to 600, which is within the range of the RBD region (i.e.,319th to 541th aa) (Figure 6). We performed molecular docking between the Furin and Spike Protein of the Omicron variant and site-specific interaction was seen at L141, V442, S443, Y446, Y486, R490, S491, S493, R495, P496, T497, Y498 and G499 (Figure 7).

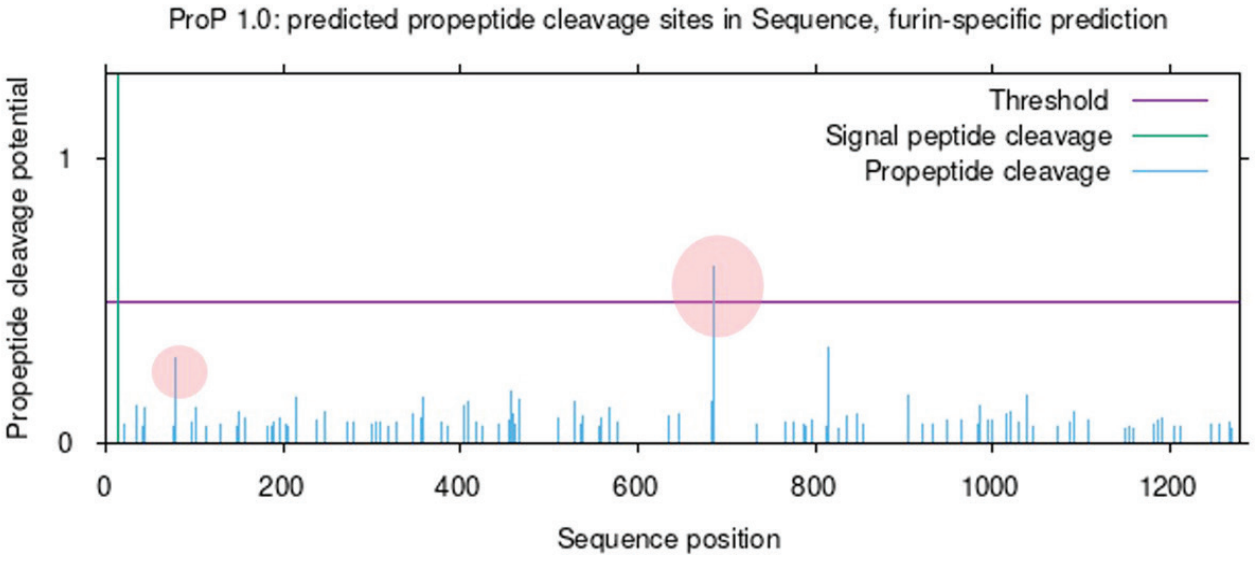


Figure 2 Graphical representation of cleavage sites (circled red) of the Sike protein of SARS-CoV-2, Wuhan variant, using ProP 1.0

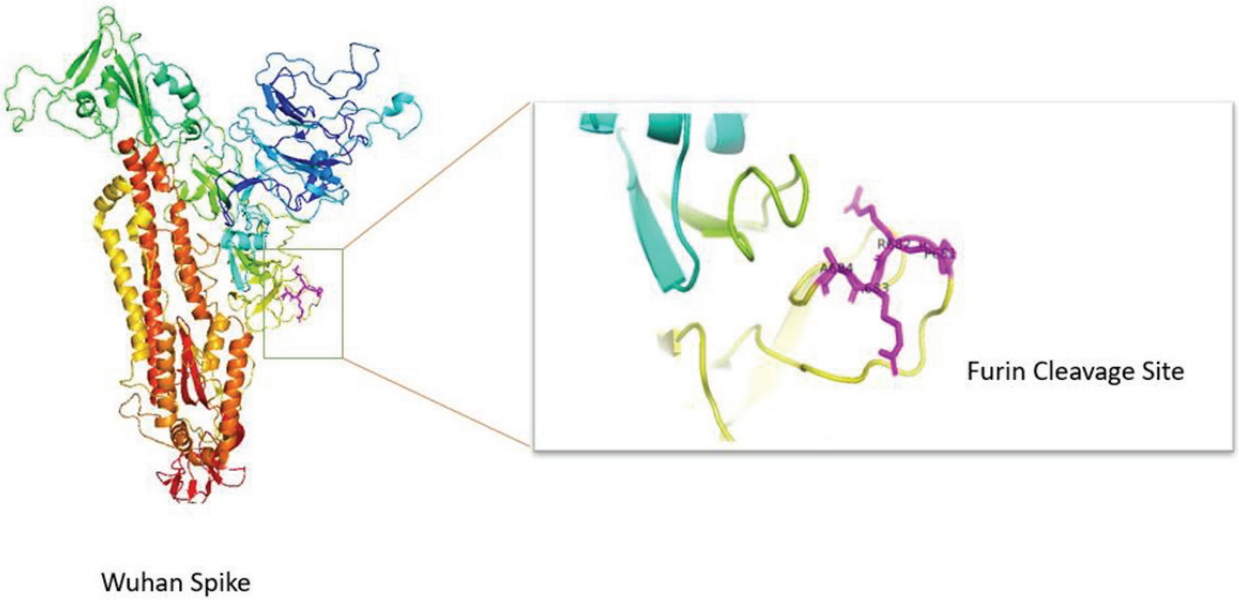


Figure 3 A diagrammatic representation of the Spike protein of SARS-CoV-2; indicating the Furin Cleavage Site

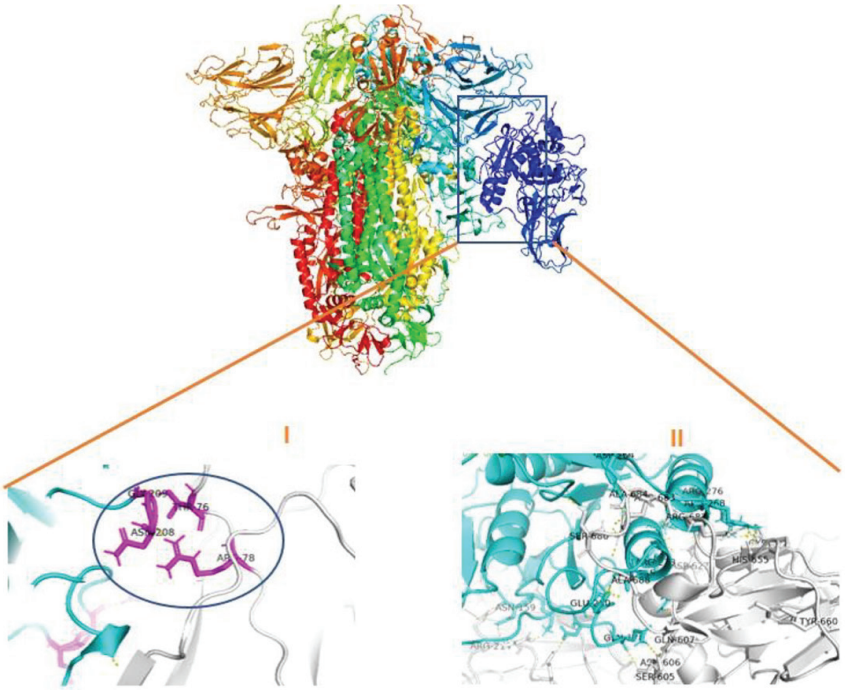
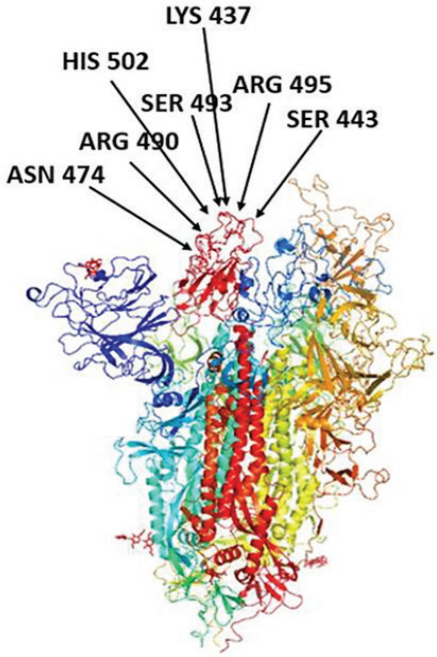
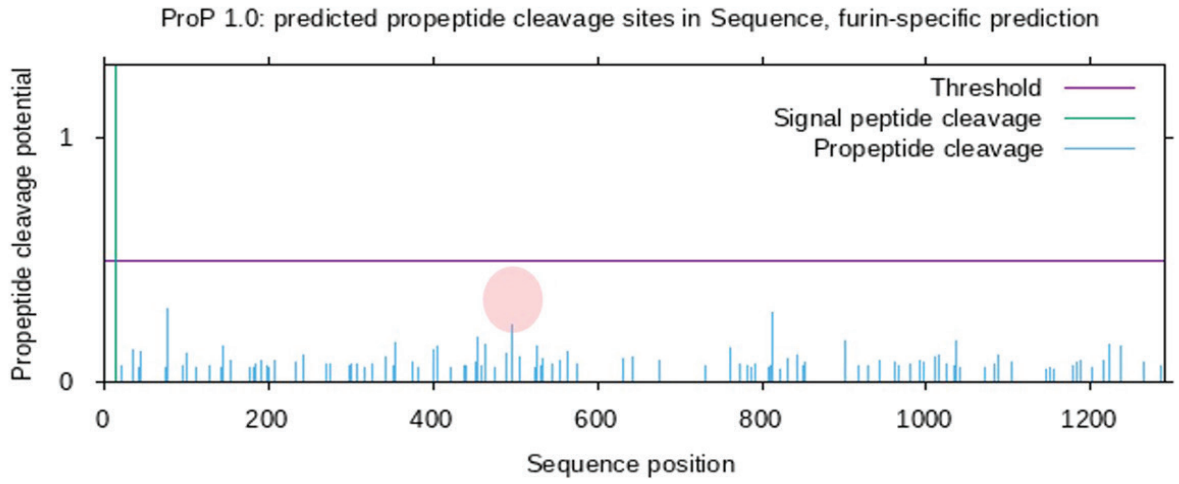


Figure 4 Diagrammatic representation depicting a closer view of the Furin cleavage site (in square brackets) in the Wuhan strain. (I). The purple amino acids are the Spike proteins; whereas, the Grey amino acids are the Furin sites. (II)



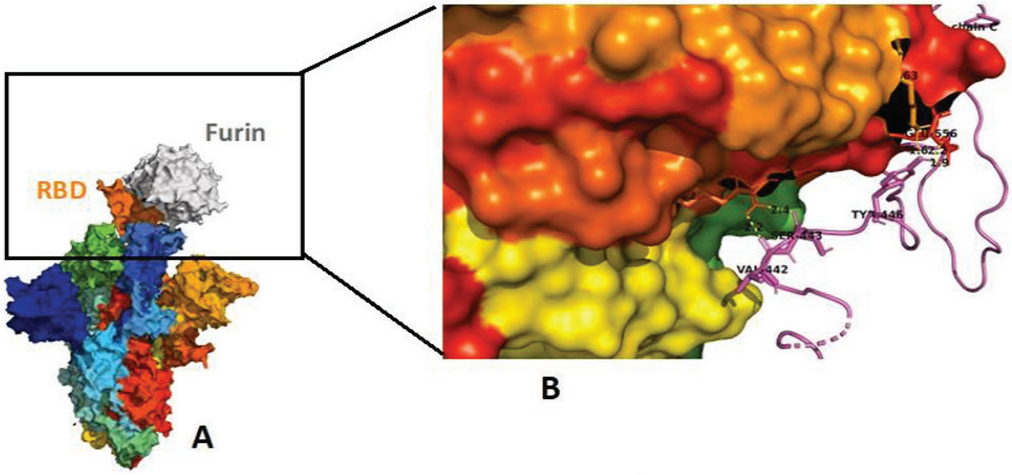
RBD=receptor binding domain, SARS-CoV-2=severe acute respiratory syndrome coronavirus 2

Figure 5 Amino acid substitutions in the RBD region of the Omicron variant of SARS-CoV-2



SARS-CoV-2=severe acute respiratory syndrome coronavirus 2

Figure 6 Graphical representation of the cleavage site of the Spike protein of SARS-CoV-2 Omicron variant using ProP 1.0



RBD=receptor binding domain

Figure 7 (A) Furin binding at the RBD region of the Omicron Variant. (B) Diagrammatic representation depicting a closer view of the furin cleavage site in Omicron. This area is also the RBD area. The purple amino acids are the Spike proteins; whereas, the Rainbow coloured amino acids are the Furin sites

Discussion

SARS-CoV-2 has been observed to continuously adapt to mutations (specifically in the S protein RBD) in order to survive, escape from the host immune response, and increase its transmission frequency. The S protein attaches to the ACE-2 receptor on human cells. With the accumulation of mutations in the Spike region of the SARS-CoV-2 from time to time, there is a possibility of multiple receptors being recognized by the new variants. Hence, besides studying the receptors of virus-host interaction biology, it is also important to study the host cell surface Proteases, as they facilitate simplification of viral protein (Spike protein in this case) as well as further insertion of the viral genome into the cytoplasm of host cells. The multiple poly-basic sites found in between the Influenza virus HA1 and HA2 domains are known to boost the virulence factor of H5 and H7 subtypes[16]. Furin is one such protease enzyme that digests basic amino acids at a sequence-specific region[9-10,17]. This present study was conducted to analyse the possible role of Furin cleavage sites for providing entry points to viral variants. Our studies indicated that in the Omicron variant, there were mutations in the receptor-binding region, and most of the amino acids were replaced by the basic amino acids; we report that the Furin will break down these amino acids and facilitate the entry of viral particles. Thus, the presence of multiple basic amino acid cleavage sites in the Spike glycoprotein of Omicron may lead to higher transmission rates compared to the other variants of SARS-CoV-2.

Currently, there are seven types of vaccine technologies that are under experiment and subsequent use to prevent infection by SARS-CoV-2. These are inactivated virus vaccines, messenger RNA (mRNA) vaccines, deoxyribose nucleic acid (DNA) vaccines, protein components, non-replicating vector viral-like particles (VLP), and live attenuated vaccines. However, as a lot

of variants have been observed since the inception of COVID-19, it is very important that a continuous evaluation of efficacies be made on these vaccines and on any drugs; including their mode of action. If drug targets could be designed based on the Furin interactive sites, and added to treatment schedules or as combinational therapy along with other anti-viral drug/vaccine molecules then we may be able to track down the entry of SARS-CoV-2.

The only limitation of this study is that our observations are based on in-silico analysis. In-vitro studies will be attempted in the future. Especially, animal model studies that are essential for establishing viral pathophysiology and correlating it with host-host transmission and infection.

Conclusion

SARS-CoV-2 recognizes more than one host receptors viz; ACE-2, Glucose-Regulating Protein 78 (GRP78), TMPRSS2, Cathepsins, Trypsins, and Furin for its possible internalization. Insertion of a polybasic site 'PRRAR' in Spike proteins S1/S2 cleavage site facilitates the recognition of Furin to cleave, initiate membrane fusion and release the viral genome into host cells. However, the mutations in Spike protein has led to the emergence of new variants, gain more affinity with host cell proteases to ensure increased transmission and pathogenesis of virus. Our study shows that the insertion of basic amino acids (LYS 437, SER 443, ASN 474, ARG 490, SER 493, ARG 495, and HIS 502) in the RBD of the Omicron variant shows higher affinity with Furin receptor compared with the other variants of concern. Thus, Furin can prove to be one of the potential therapeutic targets to block the viral entry and subsequent disease progression.

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Conflict of interest

The authors declare there are no conflicts of interest.

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