

Human interaction targets of SARS-CoV-2 spike protein: A systematic review

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Seyed Nooreddin Faraji,¹ Mohammad Javad Raei,²
Seyed Mohamad Ali Hashemi,^{3,4} Gholamreza Daryabor,⁵
Reza Tabrizi,⁶ Fateme Sadat Dashti,⁷ Emad Behboudi,⁴
Kamran Heidarnajad,⁸ Peyman Nowrouzi-Sohrabi^{9,10} and
Gholamreza Hatam¹¹

Abstract

Objectives: The development of effective targeted therapy and drug-design approaches against the SARS-CoV-2 is a universal health priority. Therefore, it is important to assess possible therapeutic strategies against SARS-CoV-2 via its most interaction targets. The present study aimed to perform a systematic review on clinical and experimental investigations regarding SARS-CoV-2 interaction targets for human cell entry. **Methods:** A systematic search using relevant MeSH terms and keywords was performed in PubMed, Scopus, Embase, and Web of Science (ISI) databases up to July 2021. Two reviewers independently assessed the eligibility of the studies, extracted the data, and evaluated the methodological quality of the included studies. Additionally, a narrative synthesis was done as a qualitative method for data gathering and synthesis of each outcome measure. **Results:** A total of 5610 studies were identified, and 128 articles were included in the systematic review. Based on the results, spike antigen was the only interaction protein from SARS-CoV-2. However, the interaction proteins from humans varied including different spike receptors and several cleavage enzymes. The most common interactions of the spike protein of SARS-CoV-2 for cell entry were ACE2 (entry receptor) and TMPRSS2 (for spike priming). A lot of published studies have mainly focused on the ACE2 receptor followed by the TMPRSS family and furin. Based on the results, ACE2 polymorphisms as well as spike RBD mutations affected the SARS-CoV-2 binding affinity. **Conclusion:** The included studies shed more light on SARS-CoV-2 cellular entry mechanisms and detailed interactions, which could enhance the understanding of SARS-CoV-2 pathogenesis and the development of new and comprehensive therapeutic approaches.

Keywords

SARS-CoV-2, COVID-19, Entry route, Spike interaction, Cellular mechanism

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¹School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran

²Center for Nanotechnology in Drug Delivery, Shiraz University of Medical Sciences, Shiraz, Iran

³Department of Bacteriology and Virology, Shiraz University of Medical Sciences, Shiraz, Iran

⁴Department of Microbiology, Golestan University of Medical Sciences, Gorgan, Iran

⁵Autoimmune Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

⁶Noncommunicable Diseases Research Center, Fasa University of Medical Sciences, Fasa, Iran

⁷Research Center for Food Hygiene and Safety, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

⁸Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran

⁹Department of Biochemistry, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

¹⁰Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran

¹¹Basic Sciences in Infectious Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Corresponding author:

Gholamreza Hatam, School of Medicine, Shiraz University of Medical Sciences, Zand Street, Shiraz 7134745794, Iran.

Email: hatamghr@sums.ac.ir



Introduction

The emerging highly pathogenic coronavirus, SARS-CoV-2, that quickly spread among adults and recently among children and adolescents has had a serious worldwide impact on human health. An accurate understanding of the global spread of viruses is critical for proper health management.^{1,2} Even though the SARS-CoV-2 virus first emerged in Asia, it has caused high rates of morbidity and mortality in Europe.³ This led to attempts for discovering the entry route and interaction targets of the virus. Evidence has indicated that the transmission risk of the new Coronavirus is modulated by demographic and clinical factors.⁴ SARS-CoV-2 can infect several human organs including the lungs, intestines, heart, liver, kidneys, brain, blood vessels, bile ducts, conjunctiva, macrophages, and T lymphocytes, and result in related manifestations, especially respiratory conditions, neurological involvement, and gastrointestinal symptoms. A challenging question is how the virus spreads throughout the body while the SARS-CoV-2 is hardly detectable in the blood. Although many infected individuals have no or mild respiratory symptoms, others develop severe pneumonia or multiple organ infection.⁵⁻⁹

SARS-CoV-2 is encompassed by a lipid bilayer, from which spike (S) protein trimers with extensive conformational flexibility protrude. The main structural protein of Coronavirus is the spike, which mediates the invasion of the virus and determines viral tissue or host tropism. Spike is cleaved into S1 and S2 components by host cell proteases such as proprotein convertase furin enzyme.¹⁰ The major function of S1 is binding to surface receptors and subsequently undergoing complete structural rearrangement, so that the S2 subunit mediates virus-cell membrane fusion and releases the virus genome into the host cell.¹¹⁻¹³ The S1 subunit of the spike is primed by TMPRSS2 or TMPRSS4 for cell adhesion. The membrane fusion of SARS-CoV-2 spike protein is mediated via the S2 subunit and the Angiotensin-Converting Enzyme 2 (ACE2) receptor.¹⁴ ACE2 is a type I transmembrane receptor and is known to be extensively glycosylated in its respective N-terminal or C-terminal ectodomains.¹⁵ Designing specific antiviral agents is an urgent need for SARS-CoV-2 infection. This antiviral development requires a clear understanding

of SARS-CoV-2 interactions with human proteins and the mutational profile of both ACE2 receptor and spike.^{16,17}

The present study aims to systematically search and report the recent findings regarding SARS-CoV-2 entry mechanisms to find the possible interactions and help researchers to design proper prophylactic or therapeutic strategies. All the interactions of the SARS-CoV-2 spike with human host cell receptors as well as proteases have been discussed.

Methods

Data source and search strategy

Two researchers independently searched for potentially eligible studies in PubMed, Scopus, Embase, and Web of Science (ISI) databases from inception to 30 July 2021 without language restriction. To identify the studies that had experimentally investigated the interaction of the SARS-CoV-2 spike and human proteins, use was made of a combination of MeSH terms and keywords including (SARS-CoV-2 OR Severe Acute Respiratory Syndrome Coronavirus 2) AND (Entry OR Route OR Mechanisms OR Receptor OR Interaction OR Attachment OR Binding) (**Appendix**). Discrepancies or disagreements between the two reviewers were resolved by a team meeting. The lists of references in the retrieved articles and previous pertinent reviews were also manually searched to minimize the likelihood of missing studies. EndNote software, version X7 was used for managing the records.

Study selection and data extraction

After removing the duplicates, three independent authors screened the search results based on title/abstract and excluded the irrelevant records. The included studies were original ones that experimentally investigated the interaction of the SARS-CoV-2 spike and human proteins and/or molecules. The studies that experimentally explored the polymorphisms or mutations of both SARS-CoV-2 spike and human targets of the spike including ACE2, TMPRSS2, furin, and other miscellaneous proteins were selected, as well. The human cell lines, tissue specimens, or any specimen from

humans were considered original and experimental studies. However, animal studies, duplicated literature, meeting abstracts without full texts, vaccine design, reports about diagnostic approaches or protocols, review studies, meta-analyses, letters, editorials, and commentaries were excluded. The following data were independently extracted by the researchers and were entered into an Excel abstraction spreadsheet: first author's name, study location, design of the study, specimens including tissues or cell lines, type of interaction, protein targets, molecular techniques, and the main results. The extracted data were rechecked by a third author for more accuracy. Disagreements on the study selection and data extraction were resolved by discussion among all researchers until a consensus was reached. After all, the extracted data were entered into an Excel file. The Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2 was applied to evaluate the methodology of the included articles as well as the risk of bias.

Statistical analysis

The frequency data of SARS-CoV-2 interaction targets for entry to human cells, applied molecular techniques for spike interaction, and used tissues

and cells for the investigation of spike interaction with human targets were presented using bar graphs in the current systematic review. Pie graphs were also used to show the countries that experimentally investigated the interaction of SARS-CoV-2 spike with human targets. However, due to the lack of the required data, no meta-analysis could be done on the effect estimates from the included studies.

Results

The primary comprehensive database search identified 5610 citations. After eliminating the duplicates, 3436 articles remained. Then, based on the initial title and abstract screening, 2789 articles were excluded due to the following reasons: irrelevant studies, review studies, book sections, vaccine or diagnostic approaches, and other reasons (letters, conference papers, and short surveys). Eventually, 647 relevant articles were retrieved for full-text review. Among these articles, 519 were removed due to having the exclusion criteria. Finally, 128 articles were selected for this systematic review (Figure 1).

The included original studies mainly used COVID-19 patients' sera, specimens, or tissues and different human tissue cell lines or animal cell lines as controls to investigate the mutations and

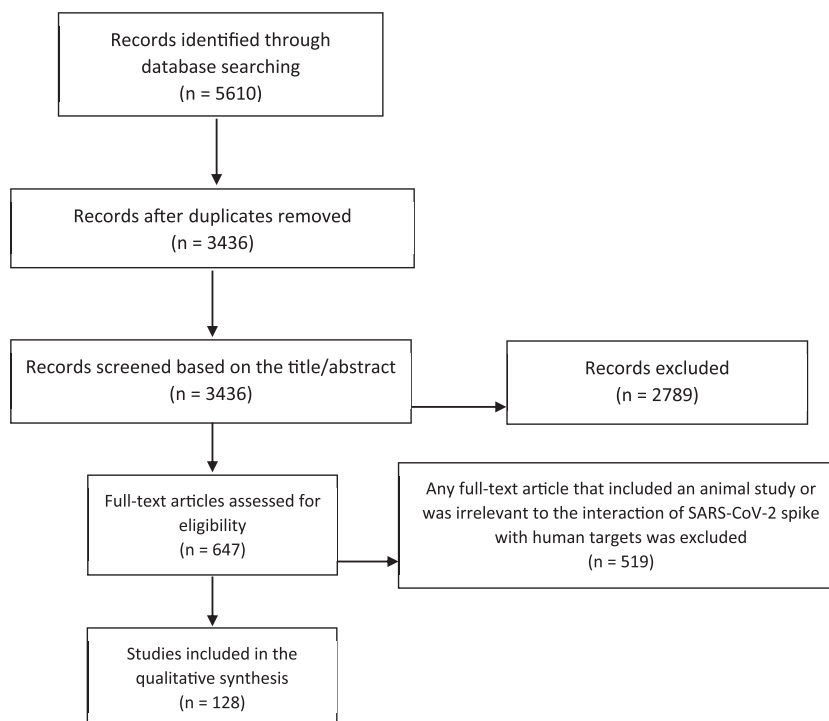


Figure 1. PRISMA flow chart of the study.

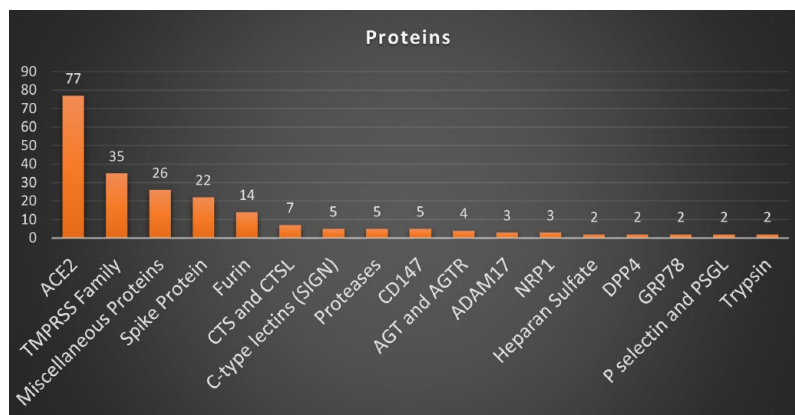


Figure 2. SARS-CoV-2 interaction targets for entry to human cells investigated in 128 studies.

polymorphisms of spike-related protein targets. Most of the included studies investigated the interaction of the SARS-CoV-2 spike as the main virus entry protein in human cells.¹⁸ The human targets of spike were ACE2 followed by TMPRSS family, furin, and other miscellaneous targets. Furthermore, several experimental techniques and different human cell lines were applied to identify the spike and human proteins. The systematic search showed that the possible interaction targets of SARS-CoV-2 investigated in these 128 studies were 49 proteins or molecules. The most investigated human protein that interacted with the SARS-CoV-2 was ACE2 followed by TMPRSS family, furin, Cathepsin (CTS), Cathepsin L (CTSL), C-type lectins, AGT, AGT receptors, and other less

evaluated proteins including CD147, proteases, ADAM17, heparan sulfate, NRP1, DPP4, 78-kDa Glucose-Regulated *Protein* (GRP78), P selectin, P selectin ligand, LY6E, trypsin, and miscellaneous proteins (Figure 2). Both SARS-CoV-2 spike/RBD mutations and ACE2 polymorphisms were investigated by deep mutational scanning and the results indicated constraints on folding and ACE2 binding. Besides, ACE2 engineering was shown to optimize binding to the spike protein, and the asparagine 90–glycosylation motif at buried sites in the interaction surface had a critical role.^{19,20} The included studies have been categorized into different protein targets (Table 1) and the main characteristics of the studies with the details have been summarized in Table 2.

Table 1. Numbers of the references working on the related proteins.

Protein	Reference
ACE2	(19–95)
TMPRSS family	(21–25, 29–33, 36–38, 41–43, 48, 54, 60, 61, 77, 83–86, 94, 96–102)
Miscellaneous proteins	(26, 29, 33, 38, 42, 84, 87, 88, 103–114)
Furin	(23, 25, 29, 34, 41, 74, 83, 96, 97, 102, 114–117)
Spike	(46, 47, 49, 51, 98, 102, 117–132)
CTS and CTSL	(26, 31, 45, 86, 102, 114, 133)
C-type Lectins (SIGN)	(77, 134, 135)
Proteases	(87, 102, 136–138)
CD147	(30, 31, 33, 139, 140)
AGT and AGTR	(33, 38)
ADAM17	(22, 60)
Heparan sulfate	(39, 141)
NRP1	(142–144)
DPP4	(22, 42)
GRP78	(31, 145)
P-selectin and PSGL	(36, 146)
Trypsin	(94, 96)

Table 2. Basic characteristics of the included studies.

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
Starr, T. N	Measuring the effect of RBD amino acid mutations on the expression of folded protein and its affinity for ACE2	Yeast cells	Deep mutational scanning, FACS, Illumina sequencing, Pseudotyped lentiviral assay, binding assays	ACE2	Most mutations were deleterious for RBD expression and ACE2 binding, while other mutations that had not been selected in the current SARS-CoV-2 pandemic isolates were well tolerated or even enhanced ACE2 binding	(19)
Chan, K. K	Analyzing the effect of ACE2 mutations on spike binding using deep mutagenesis	Fetal kidney (293T cells)	Flow cytometry, ACE2 catalytic activity assay, ELISA, virus microneutralization assay, VSV-G pseudovirus assay, spectroscopy	ACE2	Mutations in the asparagine 90-glycosylation motif and at buried sites increased affinity. The HRI sequence in the S2 possessed markedly increased alpha-helicity, thermostability, and a better binding affinity with its corresponding HR2 site	(20)
Zhou, L	Analyzing human post-mortem eyes of COVID-19 patients and surgical specimens for the expression of ACE2 and TMPRSS2	Eye tissue	Immunohistochemistry, Western blot	ACE2, TMPRSS2	IHC and WB identified the expression of ACE2 and TMPRSS2 in conjunctiva, limbus, and cornea of the eye tissue	(21)
Zhang, Q	Exploring the binding possibility of sEVs and exomeres containing ACE2 to SARS-CoV-2	Colorectal cancer (LS174T, LIM1215, DiFi, DLD-1, Caco-2, DKO-1 cell lines)	IB, FAVS, IP, Lectin Precipitation Assays	ACE2, DPP4, TACE, TMPRSS2,4	TACE, TMPRSS2, TMPRSS4, ACE2, and DPP4 were secreted in all or two/three sEVs cells. ACE2-positive DiFiEVs and exomeres could bind to the RBD, whereas sEVs and exomeres from the ACE2-negative LS174T cell line could not	(22)
L. Torre-Fuentes	Analyzing the frequency of exonic variants of the ACE2, TMPRSS2, and Furin genes in relation to the presence or absence of SARS-CoV-2 infection in a familial multiple sclerosis cohort including 120 individuals from Madrid	CNS & myelin (nerve fibers in the brain and spinal cord)	Whole-exome sequencing	ACE2, TMPRSS2, furin	ACE2 presented a low level of polymorphism, with only two variants, while TMPRSS2 was highly polymorphic and its two variants showed a significant association with SARS-CoV-2 infection. Several variants were found in the furin, none of which was associated with COVID-19	(23)
Yamamoto, M	Establishing a quantitative fusion assay dependent on SARS-CoV-2 S protein, ACE2, and TMPRSS2	Fetal kidney (Calu-3, HEK293FT, and Vero cell lines)	DSP Assay, WB, and SARS-CoV-2 infection assay	ACE2, TMPRSS2	Nafamostatmesylate significantly inhibited the fusion, while camostatmesylate was about 10-fold less active. A significantly higher dose was required for VeroE6/TMPRSS2 cells where the TMPRSS2-independent but cathepsin-dependent endosomal infection pathway likely predominated	(24)
Shang, J	Investigation of receptor binding and protease activation of the spike protein of SARS-CoV-2 by biochemical and pseudovirus entry assays	Human embryonic kidney (HEK293T cell line)	Pseudovirus entry assay, siRNA assay, WB, protein pull-down assay	hACE2, TMPRSS2, furin	The binding affinity of SARS-CoV-2 RBD and entire spike to hACE2 was higher and comparable or lower than that of SARS-CoV, respectively. This indicated that SARS-CoV-2 RBD, albeit more potent, was less exposed than SARS-CoV RBD and led to an efficient entry to the cell while evading immune surveillance. Unlike SARS-CoV, cell entry of SARS-CoV-2 was preactivated by furin	(25)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
Ou, X. Y	Identification of SARS-CoV-2 entry to host cells and spike interactions	Fetal kidney (293/hACE2 cells)	SARS-CoV-2 pseudovirus system, WB, flow cytometry, MTT, fusion assay	ACE2, PIKfyve, two-pore segment channel 2 (TPC2), CTSL	SARS-CoV-2 used ACE2 as its receptor and entered cells mainly through endocytosis. PIKfyve, TPC2, and CTSL were vital for entry. SARS-CoV-2 S protein was less stable than the S protein of SARS-CoV	(26)
Wrapp, D	Determining the cryo-EM structure of the SARS-CoV-2 S trimer in the prefusion conformation and its binding kinetics to ACE2	Kidney (FreeStyle 293 cells)	Cryo-EM, negative-stain EM, SPR sensorgram, affinity chromatography	ACE2	The spike bound ACE2 with higher affinity than did SARS-CoV S. The predominant state of the trimer had one of the three RBDs rotated up in a receptor-accessible conformation	(27)
Zhu, Y	Verification of SARS-CoV-2 interaction with human ACE2 as a cell receptor	Fetal kidney (293T cells)	Single-cycle infection assay, DSP fusion assay, N-PAGE	ACE2	Spike protein mediated high membrane fusion activity. SARS-CoV-2 might possess an enhanced HRI-HR2 interaction in comparison to SARS-CoV	(28)
Sakaguchi, W	Determining the presence of ACE2, TMPRSS2, and furin molecules in the oral cavity by examination of dorsal tongue and gingiva, saliva, and tongue coating samples	Tongue and gingival squamous epithelium, submandibular glands	IHC, RT-PCR, WB, immunofluorescence staining	ACE2, TMPRSS2, furin, N-EL	ACE2 was expressed in the stratified squamous epithelium of the dorsal tongue and gingiva. TMPRSS2 was highly expressed in the stratified squamous epithelium in the keratinized surface layer and the saliva and tongue coating samples. Furin was mainly located in the lower layer of stratified squamous epithelium and was detected in the saliva but not tongue coating samples. ACE2, TMPRSS2, and furin mRNA expressions were observed in taste bud-derived cultured cells	(29)
Qiao, J	Investigating the expression levels of receptors and related proteases for better apprehension of the neuropathy in COVID-19	Lung cancer cell line (Calu3), brain cell lines (SY5Y, HMC3, U87)	qRT-PCR, WB	ACE2, TMPRSS2, and CD147	ACE2, CD147, and TMPRSS2 were expressed in both human and mouse brain cell lines, but the expression pattern was very distinct from that of the lung. ACE2 was expressed less, but CD147 was highly expressed in brain cell lines. Mouse brain tissues were mostly compared with lung cell lines and tissues, and TMPRSS2 had a consistent expression in brain cell lines and mouse lung tissues	(30)
Aguiar, J. A	To determine the expression and in situ localization of candidate receptors in the respiratory mucosa for SARS-CoV-2	Lung (Calu-3, HBEC-6KT cells)	scRNAseq, microarray, IB, IHC, gene promoter activity analysis	ACE2, GRP78, TMPRSS2, ADAM17, CTSL-1, CD147	Low expression of ACE2 gene and absent to low promoter activity of ACE2 in a variety of lung epithelial cells. Rare ACE2 protein expression was detectable in the airway epithelium and alveoli of the human lung. TMPRSS2, CD147, and GRP78 proteins were expressed in vitro in airway epithelial cells. Broad in situ protein expression of CD147 and GRP78 in the respiratory mucosa was examined	(31)

(Continued)

Table 2. (Continued)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
Maya, E. A. L	Investigating the roles of ACE2 and TMPRSS2 genes (1273 genetic variants) in human phenotypes of general population ($n=36339$) by the 178 PheWAS technique	...	PheWAS, genotyping	ACE2, TMPRSS2	None of the gene variants reached the threshold for significance. SNPs near the TMPRSS2 genes were associated with thrombocytes count. SNPs of the ACE2 gene were associated with the use of Angiotensin II Receptor Blockers (ARBs) combination therapies as well as with the use of non-steroid anti-inflammatory and antirheumatic products	(32)
Leonardi, A	Examination of SARS-CoV-2 receptors gene expression in conjunctival epithelial cell samples and ex-vivo cornea samples using microarray or transcriptome sequencing	Cells from 18 healthy conjunctival imprints and 6 ex-vivo cornea	RNA extraction and PCR, microarray, or transcriptome sequencing	ACE2, CD147, TMPRSS2, BSG, ANPEP, AGTR2, APOBEC3A, ADAR-1	ACE2 was slightly expressed in conjunctival samples, while BSG and TMPRSS2 were expressed at intermediate levels in both the conjunctiva and cornea. ANPEP and AGTR2 were expressed at a low level in the conjunctiva. APOBEC3A and ADAR-1, two RNA editing enzymes, were involved in antiviral responses and were highly expressed	(33)
Benton, D. J	Investigating the binding of ACE2 to the furin-cleaved form of SARS-CoV-2 S by cryoEM. Classifying 10 different molecular species including the unbound, closed spike trimer, the fully open ACE2-bound trimer, and dissociated monomeric S1 bound to ACE2	Expi293F cells	Affinity chromatography, EM	ACE2, furin	The 10 structures described ACE2 binding events, which destabilized the spike trimer, progressively opening up and out the individual S1 components. The opening process reduced S1 contacts and un-shielded the trimeric S2 core, priming fusion activation and dissociation of ACE2-bound S1 monomers. The structures also revealed refolding of an S1 subdomain following ACE2 binding that disrupted interactions with S2, notably involving Asp61413-15, leading to destabilization of the structure of S2 proximal to the secondary (S2') cleavage site	(34)
Wrobel, A. G	Investigating the relationship between SARS-CoV-2 S and the RaTG13 S using cryo-EM. Investigating and comparing both furin-cleaved and uncleaved SARS-CoV-2 S. Characterizing the relative stabilities and affinities for the SARS-CoV-2 receptor, ACE2, biochemically	Expi293F	Cryo-EM, furin treatment	ACE2	Although the overall structures of human and bat virus S proteins were similar, there were key differences in their properties including a more stable pre-cleavage form of human S and about 1000-fold tighter binding of SARS-CoV-2 to the human receptor. Cleavage at the furin-cleavage site decreased the overall stability of SARS-CoV-2 S and facilitated the adoption of the open conformation, which is required for S to bind to the ACE2 receptor. Cryo-EM and functional analyses of the furin-cleaved spike from SARS-CoV-2 and the closely related spike from bat virus RaTG13 revealed differences in protein stability and binding to human receptor ACE2	(35)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
Zhang, S	Evaluating the changes in platelet and coagulation parameters in COVID-19 patients. Investigation of ACE2 expression and direct effect of SARS-CoV-2 virus on platelets	201 healthy volunteers and 589 patients suspected of having COVID-19	RNA extraction, ELISA, RT-PCR, flow cytometry, WB, EM, IF, platelet functional studies, FeCl ₃ -induced thrombus formation, thrombus formation under flow conditions ex vivo, and co-immunoprecipitation	ACE2, TMPRSS2, integrin PAC-1 binding, and CD62P (P-selectin)	Platelets expressed ACE2 and TMPRSS2. Detectable SARS-CoV-2 RNA in the bloodstream was related to platelet hyperactivity in critically ill patients. SARS-CoV-2 and its spike directly increased platelet activation such as platelet aggregation, PAC-1 binding, CD62P expression, alpha granule secretion, dense granule release, platelet spreading, and clot retraction in vitro. Spike protein enhanced the formation of thrombosis in wild-type mice transfused with hACE2 transgenic platelets	(36)
Hoffmann, M	Evaluating the SARS-CoV-2 spike interactions with ACE2 as the entry molecule and the TMPRSS2 as the priming protease	Liver (Huh-7), kidney (293T, Vero, BHK-21, MDCKII), lung and bronchus (Calu-3, MRC-5, A549, BEAS-2B, NCI-H1299), colon (Caco-2)	Pseudotyping of VSV and transduction experiments	ACE2, TMPRSS2	SARS-CoV-2 used the SARS-CoV receptor ACE2 to enter the host cell. The spike protein of SARS-CoV-2 was primed by TMPRSS2. Antibodies against SARS-CoV spike might have the ability to protect against SARS-CoV-2. A TMPRSS2 inhibitor blocked entry and might constitute a treatment option. Sera from convalescent SARS patients cross-neutralized SARS-2-S-driven entry	(37)
Ratajczak, M. Z	Investigating the expressions of ACE2 and TMPRSS2 on HSCs and EPCs cells in human umbilical cord blood	HSCs, EPCs, VSELs, MNC cells	Real-Time qPCR, FACS	ACE2, CMA1, AGTR1&2, MASI, RENIN, TMPRSS2, angiotensin 1-7, Nlrp3	ACE2 and TMPRSS2 were expressed on very small CD133 (+) CD34 (+)/Lin (-) CD45 (-) cells in human umbilical cord blood. The interaction of the ACE2 with the spike in VSELs and HSCs cells activated the Nlrp3 inflammasome, which might lead to cell death by pyroptosis, if hyperactivated. ACE2 was expressed on the surface of HSCs and VSELs of murine bone marrow, although it is known that murine cells are not infected by SARS-CoV-2	(38)
Clausen, T. M	Clarifying the interaction of SARS-CoV-2 spike protein via RBD segment with both cellular heparan sulfate and ACE2	Vero E6 or Hep3B cells, ExpiHEK cells, H1299 cells, A549, Hep3B cells, and A375 cells	TEM and NS microscopy, flow cytometry, LC-MS, IB, qPCR, pseudotyped VSV, virus plaque assays	Heparan sulfate, ACE2	Both ACE2 and heparin could bind independently to the spike in vitro, and a ternary complex could be generated using heparin as a scaffold. Heparin increased the open conformation of the RBD, which bound ACE2. Unfractionated heparin, non-anticoagulant heparin, heparin lyases, and lung heparan sulfate potently blocked spike protein binding and/or infection	(39)

(Continued)

Table 2. (Continued)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
Barnes, C. O	Determining 8 hNABs structures in complex with SARS-CoV-2 spike trimer or RBD.	Expi293F cells	X-Ray crystallography, cryo-EM, polyreactivity assays, SPR binding experiments	ACE2	Structural comparisons allowed classification into four categories: (1) VH3-53 hNABs with short CDRH3s that blocked ACE2 and bound only to "up" RBDs, (2) ACE2- blocking hNABs that bound both "up" and "down" RBDs and could contact adjacent RBDs, (3) hNABs that bounded outside the ACE2 site and recognized "up" and "down" RBDs, and (4) previously-described antibodies that did not block ACE2 and bound only "up" RBDs	(40)
Lukassen, S	Investigating the ACE2 and TMPRSS2 expression levels and their distribution across cell types in the lung tissue	Lung tissue (12 donors and 39,778 cells), bronchus (4 donors and 17,521 cells)	Single nuclei isolation and single RNA sequencing library preparation	ACE2, TMPRSS2, furin	<p> TMPRSS2 was highly expressed in both tissues in the subsegmental bronchial branches, but ACE2 was predominantly expressed in a transient secretory cell type.</p> <p>Interestingly, these transiently differentiating cells showed enrichment for pathways related to Rho GTPase function and viral processes, suggesting increased vulnerability for SARS-CoV-2 infection</p>	(41)
Zang, R. C	Analyzing the productive infection of SARS-CoV-2 in ACE2-positive mature enterocytes in human small intestinal enteroids	Enteroids from the duodenum, ileum, and colon	African green monkey kidney epithelial cell line MA104, human embryonic kidney cell line HEK293	ACE2, DPP4, ANPEP, ST14, TMPRSS2&4	The expressions of two TMPRSS2 and TMPRSS4 proteases facilitated SARS-CoV-2 spike fusogenic activity and promoted virus entry into host cells. Simulated human colonic fluid inactivated the viruses that were released into the intestinal lumen	(42)
Sacconi, A	To investigate the differences in the expressions of both ACE2 and TMPRSS2 in normal tissues from the oral cavity, pharynx, larynx, and lung tissues as well as neoplastic tissues from the same areas	Two HNSCC cell lines (Cal-27 and Detroit-562 cell lines)	RNA extraction, qRT-PCR, SARS-CoV-2 detection, NGS, IHC, TCGA, microRNA.	ACE2, TMPRSS2	<p>A significant reduction of TMPRSS2 expression in HNSCC compared to the normal tissues was observed. It was more evident in women than in men, in TP53 mutated than in wild TP53 tumors, and in HPV negative patients compared to HPV positive counterparts.</p> <p>Functionally, the multivariate effects of TP53, HPV, and other inherent variables on TMPRSS2 were modeled.</p> <p>In tumor tissues, HPV negative, TP53 mutated status, and elevated TP53-dependent Myc-target genes were associated with low TMPRSS2 expression.</p> <p>There was an anti-correlation between microRNAs and TMPRSS2 expression in SARS-CoV-2 positive HNSCC tissues. There was no TMPRSS2 promoter methylation. Tumoral tissues might be more resistant to SARS-CoV-2 infection due to the reduced expression of TMPRSS2</p>	(43)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
Allen, J. D	Impact of ACE2 glycosylation on virus binding	HEK 293F cell	Mass spectrometry, purification, SPR	ACE2	ACE2 glycan processing had a subtle influence on SARS-CoV-2 receptor recognition	(44)
Bojkova, D	Detection of viral RNA in supernatants of infected cardiomyocytes	iPS-CMs, Caco-2	RNA sequencing	ACE2- and cathepsin	Infection of cardiomyocytes in vitro in cathepsin and ACE2-dependent manner	(45)
Hörnrich, B. F	SARS-CoV-2 and SARS-CoV spike-mediated cell-cell fusion	293T, Calu-3	Western blotting, flow cytometry, RT-qPCR	S and ACE2	SARS-CoV-2 spike-mediated cell fusion differed in the expression of its receptor and proteolytic activation	(46)
Kuzmina, A	SARS-CoV-2 spike variants and convalescent or post-vaccination sera	10 human, HEK-ACE2	Neutralization assays	S and ACE2	Spike variants of SARS-CoV-2 exhibited differential neutralization resistance and infectivity to post-vaccination or convalescent sera	(47)
Müller, J. A	Infection of endocrine and exocrine pancreas cells by SARS-CoV-2	Vero E6, Calu-3, EndoC- β H1, hESC	RT-qPCR, TCID50, RNA-seq, immunoblotting, immunofluorescence	ACE2 and TMPRSS2	The pancreas as a target of SARS-CoV-2 infection	(48)
Sanders, D. W	Cholesterol function in SARS-CoV-2 entry and pathological syncytia formation	VeroE6, HEK293T, U2OS, Beas2B, Calu3, A549	TIRF imaging, heterokaryon co-culture assay, FRAP, GPMVs, pseudovirus blocking assay, pseudovirus luciferase assay, titration, SARS-CoV-2 cholesterol depletion assay, RT-qPCR, immunofluorescence	ACE2, S	SARS-CoV-2 used cholesterol for entry to cell and pathological syncytia formation	(49)
Schmitz, A	RBD-independent mechanism of SARS-CoV-2 Spike	Single-stranded DNA aptamer	DNA interaction analysis, NGS, SPR, ELONA, protein expression and purification, pulldown assays, chromatography, pseudovirus generation	ACE2	The aptamer did not block the interaction of CoV2-S with ACE2, but studies showed potent and specific pseudo viral infection inhibition by the aptamer	(50)
Suprewicz, Ł	Vimentin and SARS-CoV-2 entry	HEK293	Tissue staining, dynamic light scattering, atomic force microscopy	ACE2, S	Focusing on targeting cell host surface vimentin as a new therapeutic strategy for preventing SARS-CoV-2 infection	(51)
Yeung, M. L	Investigation of soluble ACE2 role in the entry of SARS-CoV-2	HK-2, Caco-2, A549, Calu-3, Huh7, HepG2, PLC/PRF/5, RD, HeLa, NT2, and 293T	Knockdown of gene expressions by siRNAs, high-throughput sequencing, TCID50 assay, confocal and immunofluorescence	ACE2	Angiotensin system proteins induced cell entry of SARS-CoV-2	(52)

(Continued)

Table 2. (Continued)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
Zeng, C	SARS-CoV-2 and cell-to-cell transmission	293T, Vero-E6, Vero-ACE2, Caco-2, Calu-3, NCI-H520, 293T/ACE2	Plaque assay, flow cytometry, Western blotting, neutralization assays	ACE2	SARS-CoV-2 could spread by cell-to-cell transmission	(53)
J. Kruger	Drug inhibition of SARS-CoV-2 replication in human pluripotent stem cell-derived intestinal organoids	Human intestinal organoids derived from pluripotent stem cells (PSC-HIOs)	Immunostaining	ACE2, TMPRSS2	Low doses of remdesivir efficiently inhibited SARS-CoV-2 infection and rescued PSC-HIO morphology	(54)
M. R. Bristow	Dynamic regulation of SARS-Cov-2 binding and cell entry mechanisms in remodeled human ventricular myocardium	Remodeled human ventricular myocardium	RNA-seq, microarray	ACE2	Up-regulation in ACE2 in remodeled left ventricles might explain worse outcomes in patients with COVID-19 with underlying myocardial disorders and minimize cardiac damage by counteracting ACE2 up-regulation as a possible therapeutic approach	(55)
R. Tandon	Using heparin and enoxaparin derivatives to inhibit the entry of SARS-CoV-2	HEK293T cells	Inhibitor screening, surface plasmon resonance	ACE2	Concentration-response curves proved that unfractonated heparin (UFH), enoxaparin, 6-O-desulfated UFH, and 6-O-desulfated enoxaparin could efficiently neutralize the spike expressing lentiviral vector (pLV-S) particles with different concentrations. The low serum bioavailability of intranasally administered UFH along with the data suggesting that the nasal epithelium is a portal for initial infection and transmission indicated that the intranasal administration of UFH might be an effective and safe prophylactic treatment	(56)
T. L. Pham	Elovanoid-N32 or RvD6-isomer decreased ACE2 and binding of S protein RBD after injury or INF γ in the eye	Human corneal epithelial cells	RNA-sequencing, immunohistochemistry, droplet digital PCR (dd-PCR), capillary-based Western blot, high-throughput qPCR	ACE2	The expression of the ACE2 receptor, furin, and integrins in damaged corneas or INF γ -stimulated human corneal epithelial cells (HCEC) was consistently decreased by Elovanoid (ELY)-N32 or Resolvin D6-isomer (RvD6i). There was also a concomitant decrease in the binding of spike RBD with the lipid treatments	(57)
J. M. Calandria	Downregulation of SARS-CoV-2 cell entry, canonical mediators, and enhancement of protective signaling in human alveolar cells by elovanoids	Human alveolar cells	Western blot, real-time PCR, eSight assay	ACE2	Elovanoids downregulated ACE2 and enhanced the expression of a set of protective proteins hindering cell surface virus binding and upregulating defensive proteins against lung damage. In addition, the signal of spike (S) protein found in SARS-CoV-2 infected cells was decreased by Elovanoids and their precursors	(58)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
S. A. Sheehan	Inhibition of SARS-CoV-2 infection and COVID-19 disease progression by <i>Maackia amurensis</i> seed lectin (MASL), which exerts pleiotropic actions on oral squamous cells	HSC-2 cells	RNA sequence analysis, Western blotting, transcriptional reporter assays	ACE2	MASL targeted the ACE2 receptor, decreased ACE2 expression and glycosylation, suppressed binding of the SARS-CoV-2 spike protein, and decreased the expression of inflammatory mediators by oral epithelial cells that caused ARDS in COVID-19 patients	(59)
J. Taneera	Expression profile of SARS-CoV-2 host receptors in human pancreatic islets revealed the upregulation of ACE2 in diabetic donors	Human pancreatic islets	Microarray and RNA-sequencing	ACE2, TMPRSS2, and ADAMI7	Pancreatic islets expressed ACE2, TMPRSS2, and ADAMI7 receptors irrespective of diabetes status. ACE2 expression was significantly increased in diabetic/hyperglycemic islets compared to non-diabetic ones. Islets from female donors had higher ACE2 expression than those from males. The expressions of ADAMI7 and TMPRSS2 were not affected by gender. The expressions of the three receptors were similar in young (≤ 40 years old) and old (≥ 60 years old) donors. Obese (BMI > 30) donors had higher expression levels of ADAMI7 and TMPRSS2 compared to non-obese donors (BMI < 25). TMPRSS2 expression was positively correlated to HbA1c and negatively to age, while ADAMI7 and TMPRSS2 were positively correlated to BMI. The expressions of the three receptors were similar in muscles and subcutaneous adipose tissues obtained from diabetic and nondiabetic donors. ACE2 expression was higher in sorted pancreatic β -cells compared to other endocrine cells	(60)
C. Y. Wu	Evaluation of GB-2 ability in the inhibition of ACE2 and TMPRSS2 expression by in vivo and in vitro studies	HepG2 cells and 293 T cells	Western blot analysis, quantitative real-time PCR, immunohistochemistry	ACE2, TMPRSS2	The results indicated that the expressions of ACE2 mRNA, ACE2, and TMPRSS2 protein in HepG2 and 293 T cells could be inhibited by GB-2 without cytotoxicity	(61)
K. J. Senthil Kumar	Downregulation of ACE2, a SARS-CoV-2 Spike receptor-binding domain, in epithelial cells by geranium and lemon essential oils and their active compounds	Human colorectal adenocarcinoma cell line (HT-29)	Immunoblotting, ELISA, and quantitative real-time PCR	ACE2	Geranium and lemon oils indicated significant ACE2 inhibitory effects in epithelial cells. Citronellol, geraniol, and neryl acetate were the major compounds of geranium oil and limonene that represented the major compound of lemon oil. Moreover, treatment with citronellol and limonene showed the significant downregulation of ACE2 expression in epithelial cells	(62)

(Continued)

Table 2. (Continued)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
P. Karoyan	Blockage of SARS-CoV-2 pulmonary cells infection by human ACE2 peptide-mimics	Calu-3	ELISA, biolayer interferometry experiments	ACE2	Peptide-mimics blocked SARS-CoV-2 human pulmonary cell infection with an inhibitory concentration (IC_{50}) in the nanomolar range upon binding to the virus spike protein with high affinity	(63)
K. Y. Huang	Effective blockage of viral entry and prevention of SARS-CoV-2 infection by humanized COVID-19 decoy antibody	HEK293T and H1975 cells	ELISA, Western blotting, flow cytometry, immunofluorescence staining, immunoprecipitation	ACE2	ACE2-Fc showed specific abrogation of virus replication by blocking the entry of SARS-CoV-2 spike-expressing pseudotyped virus into both ACE2-expressing lung cells and lung organoids. Furthermore, this Fc domain of ACE2-Fc activated NK cell degranulation after co-incubation with Spike-expressing H1975 cells	(64)
T. L. Ou	Attenuation of the hydroxychloroquine-mediated inhibition of SARS-CoV-2 entry by TMPRSS2	HEK293T, H1299, and H1975 cell lines	Luciferase assay	ACE2	The entry of SARS-CoV-2 into cells was more dependent on TMPRSS2 than on SARS-CoV-1. This difference was obvious when the furin-cleavage site of the SARS-CoV-2 S protein was ablated or when it was introduced into the SARS-CoV-1 S protein. Hydroxychloroquine showed efficient blockage of viral entry mediated by cathepsin L, but not by TMPRSS2, and a combination of hydroxychloroquine and a clinically-tested TMPRSS2 inhibitor prevented SARS-CoV-2 infection more potently than either drug alone	(65)
E. Prieto-Fernández	Reduction of cell attachment of SARS-CoV-2 spike protein by hypoxia through modulating the expressions of ACE2, neuropilin-1, syndecan-1, and cellular heparan sulfate	NCI-H460 human lung epithelial cells	Lactoferrin assay, flow cytometry, quantitative PCR, Western blot	ACE2	The binding of the spike to NCI-H460 human lung epithelial cells could be inhibited by hypoxia by decreasing the cell surface levels of heparan sulfate (HS), a known attachment receptor of SARS-CoV-2. This interaction was also reduced by lactoferrin, a glycoprotein that blocks HS moieties on the cell surface. The expression of syndecan-1, an HS-containing proteoglycan expressed in the lung, was inhibited by hypoxia in an HIF-1 α -dependent manner. Hypoxia or deletion of syndecan-1 resulted in the reduced binding of the RBD to host cells	(66)
P. A. C. Wing	Inhibition of SARS-CoV-2 infection of lung epithelial cells by hypoxic and pharmacological activation of HIF	Lung epithelial cells, RKO, U2-OS, Caco-2, Calu-3, U937, A549, SH-SY5Y cell lines	Immunoblotting, RT-qPCR	ACE2	Hypoxia and the HIF prolyl hydroxylase inhibitor Roxadustat reduced the expression of ACE2 and inhibited the entry and replication of SARS-CoV-2 in lung epithelial cells via an HIF-1 α -dependent pathway. Hypoxia and roxadustat inhibited SARS-CoV-2 RNA replication, showing that post-entry steps in the viral life cycle were oxygen sensitive	(67)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
M. R. Spalinger	Identification of a novel susceptibility marker for SARS-CoV-2 infection in human subjects and risk mitigation with a clinically approved JAK inhibitor in human/mouse cells	Samples from IBD patients	Western blotting, RNA isolation and qPCR, ELISA, RNA sequencing	ACE2	The autoimmune PTPN2 risk variant rs1893217 promoted ACE2 expression and increased cellular entry mediated by SARS-CoV-2 spike protein. Elevated ACE2 expression and viral entry were mediated by increased JAK-STAT signaling and were reversed by the JAK inhibitor, tofacitinib	(68)
M. Ejemel	IgA MAb blocked the interaction of SARS-CoV-2 spike and ACE2, which provided mucosal immunity	Human epithelial cells	ELISA, flow cytometry, virus neutralization assay	ACE2	MAB362, a cross-reactive human IgA monoclonal antibody that can bind to both SARS-CoV and SARS-CoV-2 spike proteins, competitively blocked hACE2 receptor binding by completely overlapping the hACE2 structural binding epitope. In addition, MAB362 IgA neutralized both pseudotyped SARS-CoV and SARS-CoV-2 in human epithelial cells expressing hACE2	(69)
Y. M. Hu	The in vitro antiviral activity of lactoferrin against common human coronaviruses and SARS-CoV-2 was mediated by targeting the heparan sulfate co-receptor	Human RD, Huh-7 cell, HEK293T cell, HCT-8 cell, Caco-2 cell, Calu-3 cell, and MRC-5 cell lines	Immunofluorescence, differential scanning fluorimetry, real-time PCR	ACE2	lactoferrin (LF) had broad-spectrum antiviral activity against SARS-CoV-2, HCoV-OC43, HCoV-NL63, and HCoV-229E in cell culture, and bovine lactoferrin (BLF) was more potent than human lactoferrin. BLF bound to heparan sulfate proteoglycans (HSPGs), thereby blocking viral attachment to the host cell. The antiviral activity of BLF could be antagonized by the HSPG mimetic heparin. The antiviral activity of LF was synergistic with remdesivir in cell culture. The N-terminal positively charged region in BLF (residues 17–41) conferred the binding to HSPGs	(70)
V. K. Outlaw	Ex vivo and in vitro inhibition of coronavirus entry by a lipid-conjugated peptide derived from the SARS-CoV-2 spike glycoprotein hrc domain	HEK293T cell	RT-qPCR, RNA sequencing	ACE2	The SARS-CoV-2 C-terminal heptad repeat (HRC)-derived lipopeptide potently blocked the spread of SARS-CoV-2 in human airway epithelial (HAE) cultures, an ex vivo model designed to mimic respiratory viral propagation in humans. While the viral spread of SARS-CoV-2 infection was widespread in untreated airways, those treated with SARS-CoV-2 HRC lipopeptide showed no detectable evidence of viral spread	(71)

(Continued)

Table 2. (Continued)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
Q. Yang	Blockage of SARS-CoV-2 viral entry upon blocking N- and O-glycan elaboration	HEK293T and Lenti-X 293 T cells	Flow cytometry	ACE2	N- and O-glycans had only minor contributions to Spike-ACE2 binding. However, these carbohydrates played important roles in regulating viral entry. Blocking N-glycan biosynthesis at the oligomannose stage using both genetic approaches and the small molecule kifunensine dramatically reduced viral entry into HEK293T cells. Blocking O-glycan elaboration also partially blocked viral entry. Mechanistic studies suggested multiple roles for glycans during viral entry. Among them, inhibition of N-glycan biosynthesis enhanced Spike-protein proteolysis	(72)
J. S. Zhang	Inhibition of SARS-CoV-2 replication by interferon-stimulated exosomal hACE2 through competitively blocking of the virus entry	HEK293T, H1299 cells	Western blot, immunofluorescence	ACE2	hACE2 existed on the surface of exosomes released by different cell types, and the expression of exosomal hACE2 was increased by IFN alpha/beta treatment. In particular, exosomal hACE2 could specifically block the cell entry of SARS-CoV-2, which led to the inhibition of SARS-CoV-2 replication in vitro and ex vivo	(73)
M. Zhong	Analyzing ACE2 and furin expression in human oral mucosa using the public single-cell sequence datasets and immunohistochemistry	Mucosal tissue from different oral anatomical sites	Single-cell sequencing analysis, immunohistochemical staining	ACE2, furin	mRNA level expression of furin enzyme and ACE2 receptor were reported in airway epithelia, cardiac tissue, and enteric canals. Immunohistochemistry results indicated that both ACE2-positive and furin-positive cells in the target tissues were mainly positioned in the epithelial layers, partly expressed in fibroblasts. Based on these findings, we speculated that SARS-CoV-2 could invade oral mucosal cells through two possible routes: First, binding to the ACE2 receptor and second, fusion with cell membrane activated by furin protease. The results showed that oral mucosa tissues were susceptible to SARS-CoV-2, which could facilitate COVID-19 infection via respiratory and fecal-oral routes	(74)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
S. Shiers	The hypothesis that ACE2 is expressed in a subset of human dorsal root ganglion (DRG) neurons was tested by using tissues obtained from organ donors or vertebrectomy surgery based on RNAscope in situ hybridization and western blot	Human dorsal root ganglion	RNAscope in situ hybridization, imaging by a confocal microscope, immunohistochemistry, Western blot, RNA sequencing	ACE2	It was shown that human DRG neurons expressed the ACE2 at RNA and protein levels. It was also demonstrated that SARS-CoV-2 and coronavirus-associated factors and receptors were broadly expressed in human DRG at lumbar and thoracic levels. ACE2 mRNA was expressed by a subset of nociceptors that expressed MRGPRD mRNA, suggesting that SARS-CoV-2 might gain access to the nervous system through entry into neurons that formed free nerve endings at the outermost layers of skin and luminal organs. Therefore, DRG sensory neurons were a potential target for SARS-CoV-2 invasion of the peripheral nervous system, and viral infection of human nociceptors might cause some of the persistent neurological effects seen in COVID-19	(75)
L. Chen	ACE2 expression was investigated in adult human hearts from healthy and diseased individuals	Human heart tissues	In-house RNA sequencing, liquid chromatography-tandem mass spectrometry (LC-MS/MS)	ACE2	Pericytes with a high expression of ACE2 might act as the target cardiac cell of SARS-CoV-2. The pericytes injury due to virus infection might result in capillary endothelial cells dysfunction inducing microvascular dysfunction. Besides, patients with basic heart failure showed increased ACE2 expression at both mRNA and protein levels, meaning that if infected by the virus, these patients might have a higher risk of heart attack and critical conditions	(76)
S. Kala	mRNA levels of ACE2, TMPRSS2, and L-SIGN were compared by qPCR in placentae from pregnant women with HIV exposed to protease inhibitor (PI)-based ART, pregnant women with HIV on non-PI-based ART, and HIV-uninfected women	Placentae	qPCR	ACE2, TMPRSS2, L-SIGN	Pregnant women of the Black race and WHIV who were on PI-based ART had a relatively lower expression of placental ACE2 compared to the White and HIV-uninfected women. This effect might potentially contribute to altered susceptibility to COVID-19 in these women	(77)
W. P. Cao	Quantification of the specific interactions between SARS-CoV-2 or SARS-CoV-1 RBD and ACE2	HEK293T	Single-molecule force spectroscopy, steered molecular dynamics (SMD)	N-linked glycan on Asn90 of ACE2	The unbinding forces between SARS-CoV-2 RBD and ACE2 range were 30–40% higher than those of SARS-CoV-1 RBD and ACE2. SARS-CoV-2 RBD interacted with the N-linked glycan on Asn90 of ACE2	(78)

(Continued)

Table 2. (Continued)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
E. E. F. Brown	Identifying and characterizing the impact of mutating certain amino acid residues in the RBD of SARS-CoV-2 and ACE2 using NanoBiT technology-based biosensor as well as pseudotyped-virus infectivity assays	HEK293T cells	Western immunoblotting, transient transfection, in vitro NanoBit assay, lentiviral pseudovirus assay	ACE2	ACE2 SNPs in the human population might account for the variability in infectivity and disease progression in the context of SARS-CoV-2 infection. Individuals harboring these SNPs (rs766996587, rs759134032, rs200909433, rs961360700) might be more resistant to the SARS-CoV-2 virus infection.	(79)
B. F. Zheng	Investigation of the potential tropism of SARS-CoV-2, with the recombinant receptor-binding subdomain I of the spike protein of SARS-CoV-2 (RBD-SDI) being used as a probe	Bone marrow cells	RT-qPCR, immunohistochemistry	ACE2	Human bone marrow cells could be strongly infected by SARS-CoV-2	(80)
X. Song	Investigating the expression level of ACE2	Alveolar macrophages, Kupffer cells within liver, and microglial cells in the brain	Flow cytometry, immunohistochemistry	ACE2	Immunohistochemistry results showed high ACE2 expressions on human tissue macrophages such as alveolar macrophages, Kupffer cells within the liver, and microglial cells in the brain at a steady state. The data suggested that alveolar macrophages, as the frontline immune cells, might be directly targeted by the SARS-CoV-2 infection. Therefore, they have to be considered for the prevention and treatment of COVID-19	(81)
C. Blume	cDNA library preparation and sequencing		Mapping, RT-PCR, qPCR, WB	Short transcript of ACE2	Short ACE2 was upregulated in response to IFN and RV16 infection, but not SARS-CoV-2 infection	(82)
P. Varma		Fetal brain	RNA sequencing, scRNA sequencing	ACE2, TMPRSS2, furin	Novel S protein interactors were abundantly present and could play a direct or indirect role in SARS-CoV-2 fetal brain pathogenesis, especially during the 2nd and 3rd trimesters of pregnancy	(83)
J. M. McCord	Examining gene expression levels	HepG2 and HPAEC	GeneChip microarray, RNA-seq assays	Nrf2, ACE2, TMPRSS2	Nrf2 activation might significantly decrease the intensity of the SARS-COV-2 cytokine storm	(84)
M. C. Johnson	Surface labeling	293FT or 293FT/ ACE2 cells	Neutralization assay	ACE2, TMPRSS2	Truncation of SARS-COV-2 spike enhanced viral pseudotyping. The D614G and R682Q modifications in SARS-COV-2 Δ 19 Spike enhanced pseudotyping efficiency	(85)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
A. Goc	Binding, fusion, and activity assay	A549	WB	ACE2, TMPRSS2, Cathepsin L	Polyunsaturated fatty acids most effectively interfered with binding to hACE2, linolenic acid, and eicosapentaenoic acid significantly blocked SARS-CoV-2 entry. Eicosapentaenoic acid showed higher efficacy than linolenic acid in reducing the activity of TMPRSS2 and cathepsin L proteases	(86)
A. Tito	Binding, fusion, and activity assay	HK-2	High-resolution mass spectrometry, Microscale thermophoresis	Pomegranate peel extract, ACE2, virus 3CL protease	Pomegranate peel extract inhibited SARS-CoV-2 spike binding to human ACE2 receptors	(87)
Y. Zhan	Cytotoxicity assay, Ca ²⁺ mobilization assay, surface plasmon resonance assay, virus entry detection	HEK293	Chromatography	Isorhamnetin, ACE2	Isorhamnetin inhibited SARS-CoV-2 spike pseudotyped virus entering ACE2(h) cells	(88)
D. Narang			Hydrogen-Deuterium Exchange Mass spectrometry (HDX-MS)	ACE2	31–41 and 353–357 protein regions of hACE2 and hACE2 binding motif of spike protein (residues 437–508) were defined as important protein-protein interfaces	(89)
K. Gorshkov	Protein conjugation to NPs; QD-spike and AuNP-ACE2, NP-based energy transfer assay	HEK293T	Quantum dots	ACE2	Endocytosis of spike bound to ACE2 was one potential mechanism for viral entry	(90)
A. Pramanik		HEK293T	Raman spectroscopy	ACE2	Antibody attached gold nanoparticles bound to SARS-CoV-2 spike protein, thereby inhibiting the virus from binding to cell receptors, which stopped virus infection and spread. It could also destroy the lipid membrane of the virus	(91)
J. Kim	mRNA transfection	Bronchoalveolar lavage	WB, immunoprecipitation	ACE2	The soluble form of hACE2 bound to SARS-CoV-2 spike protein and prevented viral entry into target cells	(92)
R. C. Larue	Investigating the SARS-CoV-2 inhibition by ACE2-derived peptides	HEK293T	Affinity precipitation assay, plaque assay	ACE2, ACE2 derived peptides	(Glu37-Gln42) ACE2 motif that is important for SARS-CoV-2 inhibition, spike-targeting ACE2-derived peptide (SAP) with the amino acid sequence "27-TFLDKFNHEAEDLFYQ-42", and other SAPs with the amino acid sequence "37-EDLFYQ-42" inhibited SARS-CoV-2 infection	(93)

(Continued)

Table 2. (Continued)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
M. Lu	Observing conformational dynamics of SARS-CoV2 spike on virus particles	HEK293T	Single-molecule fluorescence (Forster) resonance energy transfer (smFRET) imaging	ACE2, TMPRSS2, trypsin	Proteolytic processing by serine protease trypsin stimulated hACE2-dependent activation of S	(94)
J. Patiño-Galindo	Finding the evidence of a recombination event in the RBD involving ancestral lineages to both SARS-CoV and SARS-CoV-2		Sequence analyzing	hACE2	H427N and F426Y mutations had a significant effect on improving the binding affinity of RaTG13 to hACE2	(95)
Hoffmann, M	Investigating the spike cleavage site and the role of furin enzyme in S1/S2 cleavage site	Lung (Calu-3) Kidney (Vero, 293T)	Pseudotyped transduction assay, WB, syncytium formation assay	Furin, TMPRSS2, trypsin	Furin cleaved the spike at the multibasic S1/S2 site. This cleavage was essential for S-protein-mediated cell-cell fusion and entry into human lung cells. Optimizing the S1/S2 site increased cell-cell fusion, but not virus-cell fusion	(96)
Bestle, D	Analyzing the role of furin and TMPRSS2 enzymes in cleaving the spike protein	Lung (Calu-3), kidney (Vero E6, HEK293)	RNA isolation, RT-PCR, WB, IHC, microscopy	TMPRSS2, furin	Furin cleaved the spike at the S1/S2 site and the TMPRSS2 at the S29 site. TMPRSS2 was essential for the activation of spike in Calu-3 human airway epithelial cells. The furin inhibitor in human airway cells inhibited the replication of SARS-CoV-2	(97)
Kishimoto, M	Activation of SARS-CoV-2 spike protein	HEK293T, Vero E6, Vero-T2	Immunoblotting, virus entry assay, multi-cycle replication assay, indirect immunofluorescence assay	TMPRSS11D, TMPRSS13, and S	TMPRSS11D and TMPRSS13 activated the spike protein of SARS-CoV-2	(98)
Schönfelder, K	Polymorphisms of transmembrane serine protease 2 and susceptibility to SARS-CoV-2	239 SARS-CoV-2-positive and 253 SARS-CoV-2-negative	Genotyping	TMPRSS2	TMPRSS2 rs383510 polymorphism (CC genotype) was associated with an increased risk of SARS-CoV-2 infection, but was not correlated to COVID-19 severity. Neither TMPRSS2 rs2070788 nor rs12329760 polymorphisms were related to the risk of SARS-CoV-2 infection or COVID-19 severity	(99)
. B. A. Schuler	TMPRSS2 expression across the human lifespan was examined by RNA-ISH.	Human lung tissue	RNA-ISH, immunofluorescence and image acquisition	TMPRSS2	Expression of TMPRSS2 was highest in ciliated cells and type I alveolar epithelial cells (A1), and TMPRSS2 expression was increased with aging in humans. SARS-CoV-2 RNA was highly colocalized in the cells expressing TMPRSS2	(100)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
L. Fan	Magnesium treatment on methylation changes of transmembrane serine protease 2		DNA methylation	TMPRSS2	Mg treatment significantly increased 5-methylcytosine methylation at cg16371860 (TSS1500, promoter) and reduced 5-hydroxymethylcytosine levels at cg26337277 (proximity to TSS200 and the 5' untranslated region promoter)	(101)
A. Le Coupanec	Protein assay, neutralization of endosomal acidification, internalization assay, evaluation of neuroinvasiveness	CNS	WB, immunofluorescence	Cathepsins, furin, TMPRSS2, spinesin, S glycoprotein	Differences in the cleavage of the S protein and type I interferon controlled human coronavirus infection, propagation, and neuropathology within the central nervous system	(102)
Kim, S. Y	Characterization of the spike of SARS-CoV-2 and heparin-binding interactions	Porcine intestinal HP	SPR direct binding assay	Heparin GAGs	Both monomeric and trimeric forms of SARS-CoV-2 SGP bound more tightly to heparin compared to SARS-CoV and MERS-CoV SGPs. The degree and position of sulfation within HP were important for its successful binding to monomeric SARS-CoV-2 SGP.	(103)
Wang, S. B	Description of the anti-SARS-CoV-2 function of CH25H and the related mechanisms	Lung cell lines (Calu-3 cells)	Pseudovirus system, WB, RNA sequencing, binding, and fusion assay	CH25H	CH25H showed extensive anti-coronavirus activity by blocking membrane fusion. 25HC inhibited SARS-CoV-2 viral entry in human lung organoids by activating the ER-localized ACAT that consequently led to the depletion of accessible cholesterol from the plasma membrane	(104)
Farrera-Soler, L	Applying peptide array to map the antibody response of plasma from healing patients (n=12) and healthy patients (n=6)	Sf9 insect cells	RT-PCR, SARS-CoV-2 IgG serology, microarray epitope mapping, ELISA	Immunodominant linear epitopes	Three immunodominant linear epitopes were identified and two of them corresponded to key proteolytic sites on the spike protein (S1/S2 and S2'), which is critical for cellular entry. Plasma positive for the epitope adjacent to the S1/S2 cleavage site inhibited the furin-mediated proteolysis of the spike	(105)
Edwards, M. J	Analyzing the regulatory role of sphingosine in the infection of human epithelial cells by applying pseudoviral particles expressing SARS-CoV-2 spike	Nasal epithelial cells from healthy donors, Vero, HEK-293T cells	Pseudotype VSV-SARS-CoV-2, FITC-annexin V binding, TUNEL studies, flow cytometry, kinase assay	Sphingosine	Sphingosine bound to ACE2 and prevented the interaction of RBD with ACE2. Thus, exogenously applied sphingosine prevented cellular infection with pp-SARS-CoV-2 spike	(106)
Bayati, A	Sing lentivirus pseudotyped with spike glycoprotein and purified spike glycoprotein	HEK-293T and A549 cell lines	Immunoblot, confocal microscopy, endocytosis assay, purified SARS-CoV-2 spike, and pseudovirus production	Clathrin	Viral entry via clathrin-mediated endocytosis	(107)

(Continued)

Table 2. (Continued)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
S. Wang	Analyzing protein complexes interacting with SARS-CoV-2 S in pulmonary and bronchial cells by tandem affinity purification (TAP)-mass spectrometry (MS)	BEAS-2B, H1299, human lung cancer tissues, bronchoalveolar lavage fluid cells from COVID-19 patients	Western blotting, immunoprecipitation, immunofluorescence, LC and MS, tandem affinity purification, cell culture, plasmid construction, transfection, biolayer interferometry quantification assay, quantitative real-time PCR, SARS-CoV-2 virus pseudotype production, and infection	Tyrosine-protein kinase receptor UFO (AXL)	AXL was a novel candidate receptor for SARS-CoV-2, which might play an important role in promoting the viral infection of the human respiratory system. The AXL specifically interacted with the N-terminal domain of SARS-CoV-2 S. The AXL expression level was well correlated to SARS-CoV-2 S level in bronchoalveolar lavage fluid cells from COVID-19 patients. Overexpression of AXL promoted SARS-CoV-2 entry as efficiently as did the overexpression of ACE2, while knocking out AXL significantly reduced SARS-CoV-2 infection in vitro	(108)
D. P. Collins	Evaluation of specific binding of biotinylated spike and spike 1 proteins to different human cells by confocal microscopy	Multi-lineage progenitor cells (MLPC), alveolar type 2 (AT2) cells, primary human hepatocytes (PHH), and hepatocyte-like cells (HLC)	Confocal microscopy	Hepatocyte asialoglycoprotein receptor 1 (ASGr1)	The absence of ACE-2 receptors and inhibition of spike binding by an antibody to the ASGr1 on both PHH and HLC suggested that the spike protein interacted with the ASGr1. The differential antibody blocking of spike binding to AT2, PHH, and HLC indicated that the neutralizing activity of SARS-CoV-2 binding might involve additional mechanisms beyond RBD binding to ACE-2	(109)
A. Hudák	Investigation of the interaction of both SARS-CoV-2 and its spike protein S1 subunit with syndecans isoforms	K562 cells, A549	Cell culture, pseudovirus, flow cytometry, lentiviral vector system to knockdown gens, EZ4U cell proliferation assay, microscopic visualization, co-immunoprecipitation experiments	Syndecans	Syndecans, the evolutionarily conserved family of transmembrane proteoglycans, facilitated the cellular entry of SARS-CoV-2. Among syndecans, the lung abundant syndecan-4 was the most efficient in mediating SARS-CoV-2 uptake	(110)
T. Ichimura	Biopsy and autopsy kidney proximal tubules of COVID-19 patients	Lung, kidney	Immunofluorescence staining, immunohistochemistry staining, PCR and cloning, virosome assembly, WB	KIM-1/TIM-1	KIM-1 positive cells expressed less ACE2. KIM-1 was an alternative receptor to ACE2 for SARS-CoV-2. KIM-1 targeted therapeutics might prevent and/or treat COVID-19	(111)
S. Pfaender	Immune control of viral disease	Lymphocytes	Flow cytometry, WB	Lymphocyte antigen 6 complex, locus E (LY6E)	LY6E potently restricted infection by multiple CoVs including SARS-CoV and SARS-CoV-2	(112)
T. Azad	Developing a bioluminescence-based bioreporter to interrogate the interaction between the SARS-CoV-2 viral spike (S) protein and ACE2	HEK293T	Bioluminescence	Asparagine residues	Glycosylation of asparagine residues within the RBD could mediate successful viral entry/ importance of N-linked glycosylation to the RBD's antigenicity and immunogenicity	(113)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
H. Winstone	Investigating the relevance of the polybasic cleavage site in SARS-CoV-2 route of entry	Vero E6, A549	RT-qPCR, interferon assay	IFN-induced transmembrane (IFITM), furin, Cathepsin	SARS-CoV-2 was inhibited by antiviral membrane protein IFITM2 and the sensitivity was exacerbated by deleting the furin cleavage site, which restricted viral entry to low pH compartments	(114)
S. Kim		Upper respiratory system	Cloning, RT-PCR	Furin	Furin was indicated as an S cleavage site on the N-terminal of Arg685	(116)
T. Tang		Vero E6 cells	WB, fluorogenic peptide assay	Furin, S1/S2	Furin itself might not be the only protease processing the S1/S2 site. Other proteases might also be involved in this process	(117)
Bayarri-Olmos, R	ELISA-based angiotensin-converting enzyme-2/RBD inhibition assay	141 serum samples	ELISA	S	Y453F mutation increased the ACE-2 affinity and did not challenge antibody neutralization	(118)
Collier, D. A	Neutralizing antibody responses to B.1.1.7 variant following mRNA vaccination	Serum sample	Neutralizing test	S	E484K mutation in the B.1.1.7 led to a more substantial loss of neutralizing activity	(119)
Daniloski, Z	Evaluation of spike D614G mutation in SARS-CoV-2 in different human cell types	A549, HEK293FT, Huh-7.5, Calu-3, Caco-2	qPCR, flow cytometry, bio-layer interferometry, trans-complementation assay	S	The mutation of spike D614G increased SARS-CoV-2 infection	(120)
Hoffmann, M	Variants B.1.351 and P.1 of SARS-Cov2 and neutralizing antibodies	293T, 293T-ACE2, A549, A549-ACE2, A549-ACE2/ TMPRSS2, Caco-2, Calu-3, Vero, Vero-TMPRSS2	Fluorescence microscopy, qualitative and quantitative cell-cell fusion assay	S	B.1.351 and P.1 variants escaped from neutralizing antibodies	(121)
Hou, Y. J	Ex vivo replication and in vivo transmission of D614G variant	Vero-81, Vero-E6, Huh7 and A549-ACE2, HNE, LAE, SAE	Neutralization assay, immunostaining, Western blot, EM imaging, spike quantification, pathological examination	S	SARS-CoV-2 D614G variant had efficient transmission in vivo and replication ex vivo	(122)
Li, Q	Infectivity and immune escape status of SARS-CoV-2 501Y.V2 variants	Huh-7, Vero, LLC-MK2, HEK293T	RT-PCR, infection assays, neutralization assays	S	SARS-CoV-2 501Y.V2 variants had immune escape but lacked higher infectivity	(123)
Mykytyn, A. Z	SARS-CoV-2 and entry into human airway organoids	VeroE6, Vero, Calu-3	Immunofluorescence microscopy, immunohistochemistry, fusion assay, entry route assay, entry speed assay, Western blot, silver staining	S	Serine proteases helped SARS-CoV-2 to enter airway cells and the multibasic cleavage site was an adaptation to this entry strategy	(124)
Ozono, S	D614G spike mutation and entry efficiency	293T, HepG2	Cell entry assays, spike incorporation assays, biolayer interferometry, neutralization assays	S	D614G spike mutation with enhanced ACE2-binding affinity increased entry efficiency	(125)

(Continued)

Table 2. (Continued)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
Park, E. J	The spike of SARS-CoV-2 and β 1 integrins on the lung epithelial cells	C57BL/6j mice, MLO-A5, THP-1, I1-18, QG-56, MDA-MB-231, primary lung epithelial cells	Cell adhesion assay, flow cytometry, RT-qPCR	S	SARS-CoV-2 spike bound to β 1 integrins on lung epithelial cells	(126)
Sasaki, M	Generation of SARS-CoV-2 variants with mutations at the S1/S2 cleavage site	Calu-3, Vero E6, 293T	Sequencing, plaque assay, immunoblotting, indirect immunofluorescence assay, virus infection assay, deep sequencing	S	SARS-CoV-2 during propagation in TMPRSS2-deficient cells generated variants with mutations at the S1/S2 cleavage site	(127)
Zhang, L	Role of SARS-CoV-2 D614G mutation in infectivity	HEK293T, NCI-H1299, Calu-3, NCI-H1975	Purification, RT-qPCR, entry assay, SPR analyses, neutralization assay	S	D614G mutation in S protein increased virion spike density and infectivity	(128)
Zhou, B	Effect of SARS-CoV-2 spike D614G mutation on replication and transmissibility	Hamster, mouse, Vero E6, primary human nasal epithelial	BLI assay, flow cytometry, plaque assay, RT-PCR, sequencing	S	D614G variant of SARS-CoV-2 S protein conferred increased transmissibility and replication	(129)
V. Tchesnokova	Investigation of genetic variations in a 414-583 amino acid region of the spike protein, partially encompassing the ACE2 receptor-binding domain	Nasopharyngeal	PCR, RT-PCR, whole-genome sequencing	S	Increased infectivity of the L452R variants was due to leucine-452 replacement with arginine, which led to a much stronger binding to the receptor and escape from neutralizing antibodies	(130)
S. M. C. Gobeil	Exploration of S conformational changes and the effects of the D614G mutation on a soluble S ectodomain construct	293-F cells	Protein production and purification, negative-stain electron microscopy, differential scanning calorimetry, ELISA, cryoelectron microscopy	S	D614G mutation altered SARS-CoV-2 spike conformation and enhanced furin cleavage at the S1/S2 junction. Despite a low mutation rate, isolates with the D614G substitution in the S protein appeared early during the pandemic and are now the dominant form worldwide. Cryoelectron microscopy (cryo-EM) altered RBD disposition. Antigenicity and proteolysis experiments revealed structural changes and enhanced furin cleavage efficiency of the G614 variant. Furthermore, furin cleavage altered the up/down ratio of the RBDs in the G614 S ectodomain, demonstrating an allosteric effect on RBD positioning triggered by changes in the SD2 region, which harbors residue 614 and the furin cleavage site	(131)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
M. F. L. Mendoza	Monitoring viral entry using luciferase vesicular stomatitis virus producing glycoproteins	HEK293T, BHK-T7, BHK21, Vero s	Real-time PCR	S Glycoproteins	SARS-CoV-2 spike-containing rVSV particles spread poorly in multi-step replication curves and produced significantly lower titers compared to other chimeric viruses. Surprisingly, the first round of entry was comparably efficient and produced luciferase as quickly as rVSV/VSV.	(132)
M. M. Zhao	Analyzing the circulating levels of CTSL in patients with COVID-19 and systematic study of the therapeutic effects of CTSL on this disease for the first time	Huh7, A549 cells, human HEK293T cells, plasma samples	Cell culture, luciferase assay, syncytium-formation assay, real-time PCR, MTT, ELISA, pseudovirus	Cathepsin L (CTSL)	The circulating level of cathepsin L elevated after SARS-CoV-2 infection and CTSL functionally cleaved the SARS-CoV-2 spike protein and enhanced virus entry	(133)
R. Amraie	Investigation of CD209L and CD209 roles in SARS-CoV-2 entry and infection	Human lung, renal, and temporal arteriole tissues, HUVEC-TERT cells, HEK-293 cells, A549, and H1299 cells	Immunofluorescence staining, Western blot, shRNA strategy, in vitro angiogenesis assay, neutralization assays, viral entry assay, immunoprecipitation assay, Far-Western blot analysis, dot blot assay	DC-SIGN (CD209), L-SIGN (CD209L)	CD209L and CD209 served as alternative receptors for SARS-CoV-2 in disease-relevant cell types including the vascular system. Immunofluorescence staining of human tissues revealed the prominent expression of CD209L in the lung and kidney epithelium and endothelium. CD209L and CD209 interacted with S-RBD. CD209L contained two N-glycosylation sequons at sites N92 and N361, but only site N92 was occupied by CLRs, particularly DC/L-SIGN, have been associated with important steps of viral entry and infection of different viruses. The results indicated that DC/L-SIGN was an important factor contributing to additional routes of infection mediated by the S protein of SARS-CoV-2. This trans-infection process greatly facilitated viral transmission to susceptible cells	(134)
M. Thépaut	Investigation of the potential interaction of C-type lectin receptors, notably DC/L-SIGN with SARS-CoV-2, through glycan recognition of the spike envelope glycoprotein, as well as their potential role in SARS-CoV-2 transmission	Calu-3, Jurkat, blood samples	Protein production and purification, negative staining electron microscopy, surface plasmon resonance binding experiments, pseudovirus	DC/L-SIGN		(135)
S. Kamle	Because chitinase 3-like-1 induced during aging and comorbid diseases. The relationships between CHI3LI and SC2 were investigated	Human plasma samples	Western blot analysis, real-time PCR, immunohistochemistry, double label immunohistochemistry, SARS-CoV-2 pseudovirus infection, generation of monoclonal antibodies against CHI3LI, co-immunoprecipitation and immunoblot assays, generation of WT and mutant forms of recombinant CHI3LI	Chitinase 3-like-1 (CHI3LI)	CHI3LI was a potent stimulator of ACE2 and viral spike protein priming proteases (SPP). This induction was a major mechanism contributing to the effects of aging during SARS-CoV-2 infection. CHI3LI played a critical role in the pathogenesis of COVID-19 and was an attractive therapeutic target.	(136)

(Continued)

Table 2. (Continued)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
E. R. Kastenhuber	By using biochemical and cell-based assays, it was demonstrated that factor Xa and thrombin could directly cleave SARS-CoV-2 spike, enhancing viral entry	Calu3, A549, Caco2	Enzymatic assay, cell culture, plasmids and lentivirus infection, syncytia assay, Incucyte, luciferase assay, pseudovirus	Factor Xa, thrombin	Factor Xa and thrombin could directly cleave SARS-CoV-2 spike and enhance the viral entry. Anticoagulation was critical in the management of COVID-19, and early intervention could provide collateral benefit by suppressing SARS-CoV-2 viral entry	(137)
J. A. Jaimes			Fluorogenic peptide assay	Furin-like, trypsin-like, and cathepsin proteases	The S1/S2 site could be cleaved by furin-like, trypsin-like, and cathepsin proteases	(138)
K. Wang	Investigation of the possible function of CD147 in SARS-CoV-2 infection	BEAS-2B and HEK293T cell lines	Cell culture, real-time PCR, in vitro SARS-CoV-2 pseudovirus infection test, SPR assay, Co-IP assay, ELISA, electron microscopy imaging, immuno-electron microscope, fluorescence resonance energy transfer assay (FRET), hematxylin & eosin (HE) staining, multicolor immunofluorescence staining	CD147	Although ACE2 was expressed in the lung, kidney, and intestine, its expression levels were rather low, especially in the lung. The interaction between host cell receptor CD147 and SARS-CoV-2 spike protein was discovered. The loss of CD147 or blocking CD147 in BEAS-2B cell lines by anti-CD147 antibody inhibited SARS-CoV-2. Interestingly, virions were observed in the lymphocytes of the lung tissue from a COVID-19 patient. Human T cells with a property of ACE2 natural deficiency could be infected with SARS-CoV-2 pseudovirus in a dose-dependent manner, which was specifically inhibited by the anti-CD147 antibody. Furthermore, CD147 mediated virus entry into host cells by endocytosis	(139)
J. Shilts	Removing basigin from the surface of human lung epithelial cells by CRISPR/Cas9	Lung epithelial cells	CRISPR/Cas9	Basigin/CD147	No evidence for basigin/CD147 as a direct SARS-CoV-2 spike binding receptor	(140)
C. Martino	The relationship between SARS-CoV-2 susceptibility and the capacity of the human microbiome to catabolize heparan sulfate, a critical host factor involved in mediating SARS-CoV-2 infection, was investigated	HI299	Heparan sulfate modification capability, abundance, and expression by log-ratios, cultivation of <i>Bacteroides</i> strains, SARS-CoV-2 spike protein production, biotinylation, SARS-CoV-2 spike binding experiments	Glycosaminoglycan heparan sulfate	In vitro, bacterial glycosidases from unpurified culture media supernatants fully blocked SARS-CoV-2 spike binding to human HI299 protein lung adenocarcinoma cells probably by modification of the host glycosaminoglycan heparan sulfate. Thus, commensal host bacterial communities could modify HS, thereby modulating SARS-CoV-2 spike protein binding. These communities changed with age and sex of the host	(141)
L. Cantuti-Castelvetri		Olfactory epithelium	Cell culture, pseudovirus, lentiviral vector system	Neuropilin-1 (NRPI)	NRPI, known to bind furin-cleaved substrates, significantly potentiated SARS-CoV-2 infectivity	(142)

Author	Study design	Tissues/Cells	Technique	Gene/Protein	Main interaction results	Ref
J. L. Daly	Monitoring viral entry	HEK293T, HeLa, Caco-2	X-Ray crystallography	NRPI	Neuropilin-1 was a host factor for SARS-CoV-2 infection	(143)
J. Davies		Respiratory and olfactory epithelium, CNS	RNA-Seq	NRPI	Neuropilin-1 was an infection mediator for COVID-19	(144)
A. J. Carlos	GRP78 interactions with SARS-2-S and ACE2 were established by utilizing biochemical and imaging approaches	HI299	Flow cytometric analysis, immunofluorescent staining, confocal microscopy, Western blot, generation of VSV pseudotype and transduction experiments, Xtt assay, cell culture	GRP78 (a stress-inducible chaperone)	Treatment of lung epithelial cells with a humanized monoclonal antibody (hMAb159) depleted cell surface GRP78 and reduced cell surface ACE2 expression, SARS-2-S-driven viral entry, and SARS-CoV-2 infection in vitro. GRP78 directly bound to the RBD of SARS-2-S and ACE2. GRP78 was an important host auxiliary factor for SARS-CoV-2 entry and infection	(145)
S. He	Viral infectivity and attachment assay	HEK293T, Vero E6	ELISA, viral infectivity assay	P-selectin glycoprotein ligand-1 (PSGL-1)	PSGL-1 might potentially inhibit coronavirus replication in PSGL-1 + cells	(146)

sEVs: extracellular vesicles, FAVS: fluorescence-activated vesicle sorting staining, IHC: immunohistochemistry, IB: immunoblot, WB: western blot, IP: immunoprecipitation, siRNA: small interfering RNA, SPR: surface plasmon resonance, SGP: surface glycoprotein (spike), GAGs: glycosaminoglycans, HP: heparin, CTSL: cathepsin L, CH25H: cholesterol-25-hydroxylase, 25H: 25-hydroxycholesterol, ACAT: acyl-CoA: cholesterol acyltransferase, EM: electron microscopy, RBD: receptor-binding domains, DSP: dual-split-protein, N-PAGE: native polyacrylamide gel electrophoresis, HRI: heptad repeat 1, N-EI: neutrophil-elastase, scRNAseq: single-cell RNA sequencing, PhewAS: phenome-wide association scan, SNP: single nucleotide polymorphisms, HSCs: hematopoietic stem cells, EPCs: endothelial progenitor cells, VSEs: very small embryonic-like stem cells, MNC: mononuclear cells, AGTR: angiotensin II receptor type 1, TEM: transmission electron microscopy, NS: negative stain-electron microscopy, hNABs: human neutralizing antibodies, ACE2: angiotensin-converting enzyme 2, hACE2: human angiotensin-converting enzyme 2, S: spike protein, PSGL-1: P-selectin glycoprotein ligand-1, IFITM: IFN-induced transmembrane, NRPI: neuropilin-1, LY6E: lymphocyte antigen six complex, locus E, CHI3L1: chitinase 3-like-1, ASGr1: hepatocyte asialoglycoprotein receptor 1, TPC2: two-pore segment channel 2, GRP78: 78-kDa glucose-regulated protein.

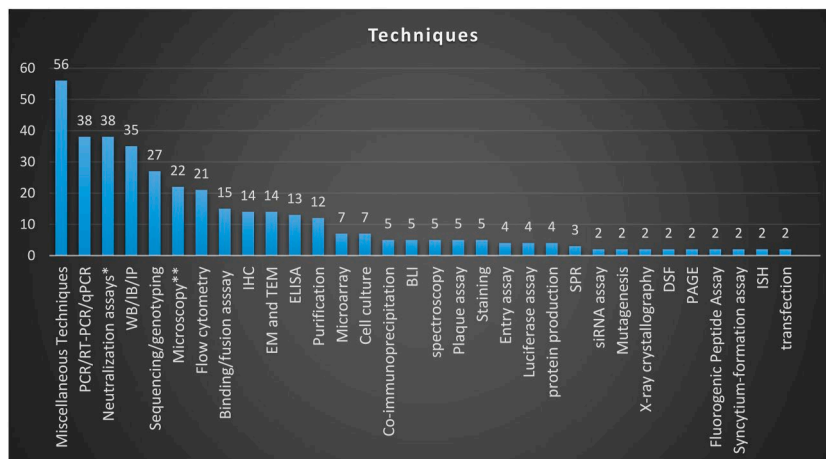


Figure 3. The molecular techniques applied for spike interaction with human targets. *, Virus/pseudovirus infection/microneutralization assay/Pseudovirus neutralization assay; **, Bright-field/confocal and immunofluorescence microscopy.

The present study aimed to explore the molecular techniques performed for the investigation of SARS-CoV-2 spike interactions with human molecules. The results were as follows: analyzing the expression of proteins by Immunoblot (IB) or Western Blot (WB) techniques in 13 studies, SARS-CoV-2 or pseudovirus/VSV-SARS-CoV-2 GFP reporter chimeric infection assay in 13 studies, RNA extraction and PCR techniques including PCR, RT-PCR, and qRT-PCR in 10 studies, binding assay techniques including DSP assay, quantitative fusion assay, biochip interaction analysis, syncytium formation assay, FITC–annexin V binding assay, and SPR binding assay in 10 studies, electron microscopy, Transmission Electron Microscopy (TEM), and negative stain-electron microscopy in eight studies, sequencing including NGS sequencing, Illumina sequencing, RNA sequencing, and whole-exome sequencing in six studies, Immunohistochemistry (IHC) and Flow Cytometry (FACS and FACS Staining) each in five studies, microarray and other microscopy techniques (Bright-field, confocal, and immunofluorescence microscopy) each in four studies, ELISA and chromatography (affinity chromatography, Pull-Down Assay (PDA) and LC-MS) each in three studies, mutagenesis (deep mutational scanning/library mutagenesis) in two studies, and other miscellaneous techniques (Appendix and Figure 3).

These molecular techniques were performed on several human tissues as well as on human or animal cells as negative controls. Kidney tissues

or cells from human or animal models were employed in almost all studies. Embryonic kidney tissue or cells (HEK293FT or Expi293F cells) were used in 17 studies, African green monkey kidney (Vero E6, MA104 cell) were utilized in eight studies, and baby hamster kidney cells (BHK-21/WI-2 cells) were used in two studies. The next most common tissue or cell line was the respiratory tract organ. Lung or bronchial tissues or cells (Calu-3, Cal-27, Detroit-562, A549, BEAS-2B, NCI-H1299, MRC-5, and HBEC-6KT) were used in 11 studies. Gastrointestinal tract or colorectal tissues or cell lines (LS174T, LIM1215, DiFi, DLD-1, Caco-2, and DKO-1) were applied in six studies and liver (Huh-7, Hep3B) was used in three studies. Other organs and tissue cells were as follows: Hematopoietic and Endothelial Cells (HSCs), eye (conjunctiva, limbus, and cornea), and CNS (myelin)/brain cell lines (SY5Y, HMC3, and U87) each in two studies and oral cavity tissues (tongue squamous epithelium, gingival squamous epithelium, and submandibular glands), cervical cancer (Hela cells), Madin-Darby canine kidney (MDCKII), Very Small Embryonic-Like Stem Cells (VSELs), Mononuclear Cells (MNC), Umbilical Cord Blood (UCB), and melanoma (A375) each in one study (Figure 4). Moreover, 13 studies were conducted in the USA, four studies in China and Germany, two studies in Japan, Spain, and Italy, and one study in the UK, Canada, Switzerland, and the Netherlands (Figure 5).

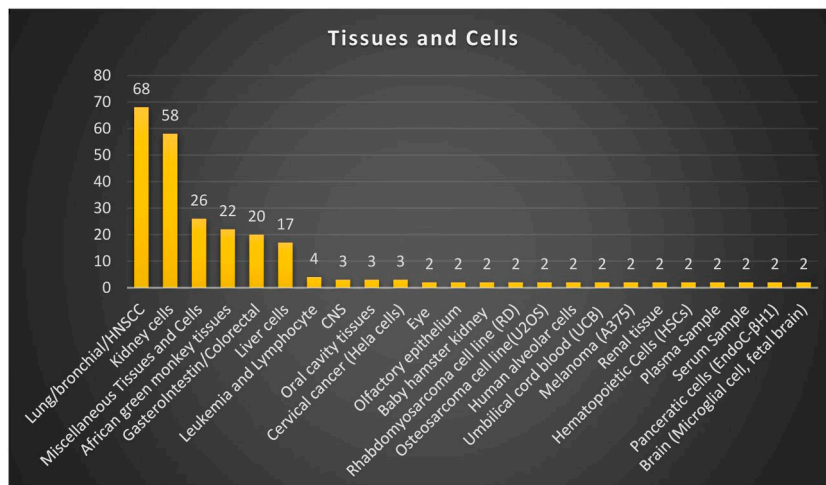


Figure 4. Human or animal tissues and cells used for the investigation of spike interaction with human proteins.

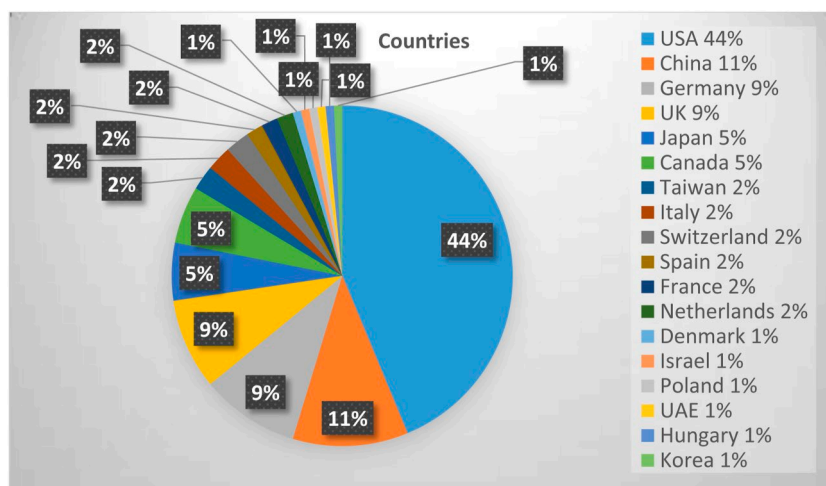


Figure 5. Names and percentages of the countries that experimentally investigated the interaction of SARS-CoV-2 spike with human targets.

Discussion

A key target of SARS-CoV-2-related therapeutic efforts is the metastable spike (S) protein with several conformational structures such as postfusion state before and after attachment to target cells.^{147,148} The wild-type S protein was shown to be heavily glycosylated, contained three hinges in the stalk domain for head orientational flexibility, and occurred mostly in the closed prefusion conformation.¹⁴⁹ Shang et al. investigated the receptor binding of SARS-CoV-2 spike compared to SARS-CoV and found that SARS-CoV-2 RBD was more potent and less exposed, had higher hACE2 binding

affinity compared to SARS-CoV RBD, and subsequently had efficient cell entry. SARS-CoV-2 cell entry was also less dependent on target cell proteases and was directly preactivated by furin.²⁵ The most important target proteins that interacted with spike protein were ACE2 and TMPRSS2.¹⁵⁰ In this systematic search, several potential SARS-CoV-2 interaction targets including receptors, proteases, and cofactors were suggested, as well. However, some of the reported targets were not investigated in human tissues. These alternative targets included furin,^{151,152} TMPRSS4,^{153–154} cathepsin L or CTSL^{155,156} as a key protease, CD147,^{157–159} Neuropilin-1 or NRP1,¹⁶⁰ angiotensin II receptor

type 2 or AGTR2,³³ C-type lectins (L-SIGN and DC-SIGN)⁷⁷ as receptors, and ADAM17.¹⁶¹ Some authors divided these targets into two groups of conventional (ACE2, TMPRSS2, ADAM17, and CTSL) and non-conventional (CD147 and GRP78) SARS-CoV-2 receptor genes. Lymphocyte antigen six complex, locus E (LY6E), was surprisingly reported to restrict the infection by SARS-CoV-2.¹¹² CD147-spike protein was a novel route for SARS-CoV-2 infection to the host cells and NRP1 was a new potential SARS-CoV-2 infection mediator implicated in COVID-19 neurological features and central nervous system involvement.^{159, 160}

Different expression patterns of ACE2 and other related proteins would change the cellular entry of SARS-CoV-2. The interaction of spike protein with human targets varied in different organs or tissues. For instance, the new virus had a different way of infection to the cerebral nervous system.^{30, 162} In addition, Descamps et al. revealed that ACE2 expression, particularly in sinuses, vocal cords, salivary glands, and oral cavity epithelial cells, was enriched in COVID-19 specimens.¹⁶³ While the oral cavity with abundant essential molecules for SARS-CoV-2 infection might be the entry route for SARS-CoV-2, other factors including protease inhibitors in the saliva that block viral entry should be considered.²⁹ It has been reported that in the gastrointestinal tissue, ACE2 is highly expressed and processed by the activity of Tumor Necrosis Factor-Alpha Converting Enzyme (TACE) and TMPRSS2.²² SARS-CoV-2 may also infect the early embryo, because NRP1 has been reported to be rich in trophoblasts. Besides, the expressions of ACE2 and TMPRSS2 in these tissues raise the possibility of vertical transmission.¹⁶⁴ In human pancreatic islets, ACE2, ADAM17, and TMPRSS2 were expressed irrespective of diabetes status.⁶⁰ The use of eyes as an entry route and the presence of SARS-CoV-2 in tears and conjunctival secretions have also become controversial. To address this issue, Zhou et al. investigated human post-mortem eyes as well as surgical specimens to find whether ocular surface cells possess the key factors (ACE2, TMPRSS2) required for cellular susceptibility to SARS-CoV-2 infection. They revealed that ACE2 and TMPRSS2 were expressed in several eye tissues, suggesting that conjunctival cells of the ocular surface cells were susceptible to infection by SARS-CoV-2.²¹ Similarly, Leonardi

et al. revealed the ocular surface as an entry point and concluded that the conjunctiva and the cornea could adopt antiviral countermeasures, which might explain the low prevalence of eye involvement.³³ However, Jianhua et al. came to almost different results.¹⁶⁵ Zhang et al. also showed that the platelets in COVID-19 patients expressed ACE2, and TMPRSS2 and SARS-CoV-2 directly enhanced thrombosis formation.³⁶ In the same line, Wang et al. disclosed the expression of the ACE2 in EOL-1 cells and found that the expression of ACE2 protein in human lung and bronchial-related cells was higher than that in HEK293T cells.¹⁶⁶

It has also been claimed that some individuals in different geographical regions had a genetic susceptibility to SARS-CoV-2 infection via polymorphism in human genes related to SARS-CoV-2 entry, especially in the ACE2 and TMPRSS2 genes. TMPRSS2 is highly polymorphic.¹⁶⁷ In the present study, the human genes and variants investigated regarding SARS-CoV-2 spike interaction included ACE2 (rs17264937, rs5980163, rs4646190, and rs4646156) and TMPRSS2 (rs150965978, rs28401567, rs457274, rs9975623, rs75603675, rs61735792, and rs61735794). Torre-Fuentes et al. explored the variants of these genes in 120 individuals with familial multiple sclerosis from Madrid through whole-exome sequencing and found that some variants, especially in TMPRSS2, might be associated with COVID-19 disease. The ACE2 gene showed a low level of polymorphism, and none of the variants was significantly associated with SARS-CoV-2 infection. Moreover, the rs75603675 position of TMPRSS2 was reported to be associated with SARS-CoV-2 infection. Nonetheless, the variants of TMPRSS2 were shown not to coincide with those described in other studies. The synonymous variants rs61735792 and rs61735794 had a significant association with the infection.²³ Furthermore, population genetic analysis indicated that G amino acid in the 614 position prevented loop disorder in spike trimer, which subsequently led to selective advantage and made a dominant form. This added interaction appeared to prevent the premature dissociation of the G614 trimer, effectively increasing the number of functional spikes, enhancing infectivity, and modulating structural rearrangements for membrane fusion. Besides, the G614 variant grew to a higher titer in

pseudotyped virions. In infected individuals, G614 was associated with higher upper respiratory tract viral loads, but not with increased disease severity. Additionally, recurrent deletions in the SARS-CoV-2 spike glycoprotein led to antibody escape.^{168–172} In addition to D614G, variants including A475V, L452R, V483A, and F490L became resistant to some neutralizing antibodies.¹⁷³ Another researcher referred to glycan structures on a recombinant SARS-CoV-2 S immunogen. This analysis enabled the mapping of the glycan-processing states across the trimeric viral spike.¹⁷⁴ The crystal structure at 2.9 Å resolution of the RBD bound with the peptidase domain of human ACE2 showed that the RBD presented a gently concave surface, which cradled the N-terminal lobe of the peptidase. The atomic details at the interaction interface clarified the importance of residue changes that facilitated efficient human-to-human transmission.¹⁷⁵

While infection by SARS-CoV-2 is spreading rapidly worldwide, no drug has been effective for treating COVID-19. Most of the proposed medications have been divided into the inhibitors of SARS-CoV-2 cell entry including ACE2 and TMPRSS2 and inhibitors of replication, membrane fusion, and assembly of SARS-CoV-2.¹⁷⁶ Therefore, by evaluating spike interaction targets defined in the present study, a considerable therapeutic approach based on the virus entry mechanisms may be developed for the treatment of COVID-19. Nafamostat mesylate that previously blocked MERS-CoV S protein-mediated cell fusion by targeting TMPRSS2 potently inhibited SARS-CoV-2 cell fusion, which made it a likely candidate drug to treat COVID-19.²⁴ Recombinant human ACE2 protein and anti-spike Monoclonal Antibodies (mAb) also inhibited SARS-CoV-2 spike protein-induced platelet activation.³⁶ Other promising medications included chloroquine and hydroxychloroquine as ACE2 blockers,¹⁶⁶ mAb against spike or RBD,⁴⁰ furin inhibitor (MI-1851), and a combination of various TMPRSS2 inhibitors with furin inhibitor (MI-1851) that produced more potent antiviral activity against SARS-CoV-2.⁹⁷

The limitation of the current study was that a meta-analysis of the effect estimates from the included studies was not possible, because of the significant heterogeneity across the included studies.

Conclusion

Understanding the details of biological interactions and underlying SARS-CoV-2 entry mechanisms is of utmost importance while designing new therapeutic approaches. In the present study, several spike interaction proteins were identified via searching original human studies. The results clarified that the SARS-CoV-2 virus entered the cells via ACE2 as the main receptor and TMPRSS2 as the main cleavage enzyme. The potential inhibitors of these pathways are currently available and have shown promising results. However, larger clinical studies are required to reveal the safety and efficacy of these medications.

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Author contribution statement

Prof. Golamreza Hatam and Dr Faraji conceived of the presented idea. Dr Faraji and Dr Tabrizi developed the theory and Dr Noruzi performed the search strategy. Dr Faraji encouraged Dr Raei, Dr Daryabor, Mr. Hashemi, and Mrs. Dashti to investigate the search results and supervised the findings of this work. Mr. Behboudi gathered the included studies and summarized them. Dr Tabrizi verified the analytical methods and Mr. Heidarnajad rechecked the results and critically revised the manuscript. All authors discussed the results and contributed to the final manuscript.

Declaration of conflicting interests

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Ethics approval


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Informed consent

This is a review article, animal and human experiments have not participated

ORCID iDs

Fateme Sadat Dashti  <https://orcid.org/0000-0002-2144-954X>

Emad Behboudi  <https://orcid.org/0000-0002-8971-0775>

Kamran Heidarmejad  <https://orcid.org/0000-0002-5962-9052>

Gholamreza Hatam  <https://orcid.org/0000-0002-7807-4793>

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Appendix

Abbreviations

SARS-CoV-2
severe acute respiratory syndrome coronavirus 2
MeSH
medical subject headings
ACE2
angiotensin-converting enzyme 2
TMPRSS
transmembrane serine protease
S
spike
RBD
receptor-binding domains
AGT
angiotensinogen
AGTR
angiotensin receptor type 1
ADAM17
A disintegrin and metalloprotease 17
TACE
Tumor Necrosis Factor-Alpha Converting Enzyme
NRP1
Neuropilin 1
DPP4
dipeptidyl peptidase four
GRP78
78-kDa glucose-regulated protein
LY6E
lymphocyte antigen six complex, Locus E
siRNA
small inhibitory RNA

sEVs
extracellular vesicles
IHC
immunohistochemistry
WB
Western blot
IB
immunoblot
IP
immunoprecipitation
WES
whole-exome sequencing
DSP assay
dual split-protein assay
FAVS
fluorescence-activated vesicle sorting staining
N-PAGE
native polyacrylamide gel electrophoresis
EM
electron microscopy
TEM
transmission electron microscopy
NS
negative stain-electron microscopy
PDA
photodiode-array detection
LC-MS
liquid chromatography-mass spectrometry
DSF
differential scanning fluorimetry
ISH
in situ hybridization
BLI
bio-layer interferometry
SPR
surface plasmon resonance
SGP
surface glycoprotein (spike)
GAGs
glycosaminoglycans
HP
heparin
CTSL
cathepsin L
CTS
cathepsin
CH25H
cholesterol-25-hydroxylase
25H
25-hydroxycholesterol
ACATacyl-CoA
acyl-CoAcholesterol acyltransferase
HR1
heptad repeat 1
N-EI
neutrophil-elastase
scRNAseq
single-cell RNA sequencing
PheWAS
phenome-wide association scan
SNP
single nucleotide polymorphisms
HSCs
hematopoietic stem cells
EPCs
endothelial progenitor cells
VSELs
very small embryonic-like stem cells
MNC
mononuclear cells
UCB
umbilical cord blood
hNAbs
human neutralizing antibodies
hACE2
human angiotensin-converting enzyme 2
PSGL-1
P-selectin glycoprotein ligand-1
IFITM
IFN-induced transmembrane
CHI3L1
chitinase 3-like-1
ASGr1
hepatocyte asialoglycoprotein receptor 1
TPC2
two-pore segment channel 2