Host cell entry mediators implicated in the cellular tropism of SARS-CoV-2, the pathophysiology of COVID-19 and the identification of microRNAs that can modulate the expression of these mediators (Review)

PERIKLIS KATOPODIS[1,2*], HARPAL S. RANDEVA[3,4], DEMETRIOS A. SPANDIDOS[5], SAYEH SARAVI[1], IOANNIS KYROU[3,4,6-8*] and EMMANOUIL KARTERIS[1,2*]

[1]Biosciences, College of Health, Medicine and Life Sciences, Brunel University London, Uxbridge UB8 3PH; [2]Division of Thoracic Surgery, The Royal Brompton and Harefield NHS Foundation Trust, Harefield Hospital, London UB96JH; [3]Warwickshire Institute for The Study of Diabetes, Endocrinology and Metabolism (WISDEM), University Hospitals Coventry and Warwickshire NHS Trust, Coventry CV2 2DX; [4]Warwick Medical School, University of Warwick, Coventry CV4 7AL, UK; [5]Laboratory of Clinical Virology, Medical School, University of Crete, 71409 Heraklion, Greece; [6]Centre for Sport, Exercise and Life Sciences, Research Institute for Health and Wellbeing, Coventry University, Coventry CV1 5FB; [7]Aston Medical School, College of Health and Life Sciences, Aston University, Birmingham B4 7ET, UK; [8]School of Food and Nutritional Sciences, Department of Food Science and Human Nutrition, Agricultural University of Athens, 11855 Athens, Greece

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Abstract. The pathophysiology of coronavirus disease 2019 (COVID-19) is mainly dependent on the underlying mechanisms that mediate the entry of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) into the host cells of the various human tissues/organs. Recent studies have indicated a higher order of complexity of the mechanisms of infectivity, given that there is a wide-repertoire of possible cell entry mediators that appear to co-localise in a cell- and tissue-specific manner. The present study provides an overview of the 'canonical' SARS-CoV-2 mediators, namely angiotensin converting enzyme 2, transmembrane protease serine 2 and 4, and neuropilin-1, expanding on the involvement of novel candidates, including glucose-regulated protein 78, basigin, kidney injury molecule-1, metabotropic glutamate receptor subtype 2, ADAM metallopeptidase domain 17 (also termed tumour necrosis factor-α convertase) and Toll-like receptor 4. Furthermore, emerging data indicate that changes in microRNA (miRNA/miR) expression levels in patients with COVID-19 are suggestive of further complexity in the regulation of these viral mediators. An *in silico* analysis revealed 160 candidate miRNAs with potential strong binding capacity in the aforementioned genes. Future studies should concentrate on elucidating the association between the cellular tropism of the SARS-CoV-2 cell entry mediators and the mechanisms through which they might affect the clinical outcome. Finally, the clinical utility as a biomarker or therapeutic target of miRNAs in the context of COVID-19 warrants further investigation.

Correspondence to: Dr Emmanouil Karteris, Biosciences, College of Health, Medicine and Life Sciences, Brunel University London, Kingston Lane, Uxbridge UB8 3PH, UK
E-mail: emmanouil.karteris@brunel.ac.uk

Dr Ioannis Kyrou, Warwickshire Institute for The Study of Diabetes, Endocrinology and Metabolism (WISDEM), University Hospitals Coventry and Warwickshire NHS Trust, Clifford Bridge Road, Coventry CV2 2DX, UK
E-mail: kyrouj@gmail.com

*Contributed equally

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV2, severe acute respiratory syndrome coronavirus 2; ACE2, angiotensin converting enzyme 2; NPR1, neuropilin-1; TMPRSS2 and 4, transmembrane protease serine 2 and 4; ADAM17, ADAM metallopeptidase domain 17 (also termed tumour necrosis factor-α convertase); KIM1, kidney injury molecule-1; TLR4, Toll-like receptor 4; GRP78, glucose-regulated protein 78; miRNAs, microRNAs

Key words: COVID-19, SARS-CoV2, ACE2, NPR1, TMPRSS2, TMPRSS4, ADAM17, TLR4, GRP78, miRNAs

Contents

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped RNA virus that is transmitted mainly via air droplets, and can cause a viral infectious disease in humans, namely coronavirus disease 2019 (COVID-19), which was first identified towards the end of 2019 (1). COVID-19 manifestations range from mild (asymptomatic or mild respiratory tract infection in the majority of reported cases) to severe and the disease can be fatal in high-risk individuals, with a spectrum of respiratory and/or extra-pulmonary manifestations that may require hospitalization and potentially mechanical ventilation and intensive care unit support (1). Of note, SARS-CoV-2 is highly virulent, has a tendency to mutate and exhibits a cellular tropism within the human body that appears to be associated with the expression of various host cell entry mediators, such as the angiotensin converting enzyme 2 (ACE2) (2-4). The present review article presents a concise overview of the key host cell entry mediators which have been identified to play a role in the infection of human cells by SARS-CoV-2, summarizing the evidence on the mechanisms through which ACE2 and additional host receptors/proteins appear to be implicated in the cellular tropism of SARS-CoV-2 and the pathophysiology of COVID-19. Moreover, the present review article aims to provide insight into the mechanisms through which these cell entry mediators may be regulated by certain microRNAs (miRNAs/miRs).

2. Angiotensin converting enzyme 2, and transmembrane protease serine 2 and 4

Both SARS-CoV and SARS-CoV-2 have been reported to use ACE2 as the key receptor for their host cell entry (4-6). Indeed, an initial step in these viral infections is the binding of this cellular receptor with the viral spike S glycoprotein (S protein), which consists of the S1 and S2 subunits (Fig. 1). The S1 subunit contains a C-terminal receptor-binding domain that recognizes and binds to the specific host receptor (e.g., ACE2), whilst the S2 subdomain is responsible for the fusion of the viral membrane with the host cell membrane via the fusion peptide. Following this binding to ACE2, a protease cleavage of the S protein follows. This process is catalysed by transmembrane protease serine (TMPRSS)2, and other host proteases, such as cathepsin L and furin at the S1-S2 site, which activate the entry of SARS-CoV-2 (5,7-11).

Based on consistent data, ACE2 is now well-established as the key mediator that facilitates the entry of SARS-CoV-2 into human host cells (6,12,13). Notably, ACE2 is highly expressed in the kidneys, small intestine, prostate and lung alveolar epithelial type II cells, which explains the documented tropism of this novel coronavirus and the related pulmonary and extrapulmonary symptoms that a SARS-CoV-2 infection may cause (14). The analysis of autopsy tissue from fatal COVID-19 cases has most frequently detected SARS-CoV-2 RNA in the airway epithelium, whilst SARS-CoV-2 RNA has also been found to be highly co-localised in cells expressing TMPRSS2 (15-18). Of note, it has been suggested that DNA polymorphisms for ACE2 and/or TMPRSS2 may be associated with the genetic susceptibility of different populations to severe COVID-19 infection (9). Along with TMPRSS2, TMPRSS4 has also been shown to be involved in the infectivity of SARS-CoV-2. In human small intestinal enterocytes, the presence of both enzymes drives the cleavage of the spike S glycoprotein of SARS-CoV-2 and enhances subsequent membrane fusion (19). Moreover, TMPRSS2 and TMPRSS4, along with ACE2, are abundantly co-expressed in the small intestine and colon (20). Subsequently, the authors have previously demonstrated that TMPRSS4 is overexpressed in 11 types of cancer in the colon mucosa and in all the brain regions that are associated with the sense of taste and smell (21).

3. Neuropilin-1

It is noteworthy that the relatively low/moderate expression of ACE2 in the human respiratory system has triggered further research focusing on the discovery of potential additional cell proteins/receptors that may mediate the SARS-CoV-2 infectivity and contribute to the selective tissue/organ tropism of this new coronavirus (22,23). In this context, two elegant studies by Daly *et al* (24) and Cantuti-Castelvetri (25) *et al* have demonstrated that neuropilin (NRP)1 constitutes an additional cellular mediator implicated in the entry of SARS-CoV-2 into human cells. Indeed, based on the experimental data of both these studies, it is evident that, not only ACE2, but also NRP1 can act as a host cell mediator that enhances the infectivity of SARS-CoV-2 and contributes to its documented tissue/organ tropism.

Overall, NRPs are classed as non-tyrosine kinase, single-transmembrane glycoproteins which are greatly conserved in vertebrates and constitute co-receptors for a number of molecules, such as semaphorins and vascular endothelial growth factors (VEGFs), playing a key role in numerous physiological processes (e.g., neuronal development and axon control, immune function angiogenesis, cell proliferation and vascular permeability) (23-26).

Of note, the aforementioned studies by Daly *et al* (24) and Cantuti-Castelvetri *et al* (25) suggest that, unlike SARS-CoV S proteins, SARS-CoV-2 S proteins have a polybasic sequence motif (Arg-Arg-Ala-Arg) at the S1-S2 junction/boundary that conforms to the 'C-end rule' (CendR motif) [R-XX-R; where R denotes arginine (Arg), can be replaced by lysine (Lys)] and serves as a cleavage site for the convertase furin (Fig. 1) (24-26). Thus, SARS-CoV-2 can enter host cells more easily with the aid of NRP1, boosting its infectivity and promoting its tropism (24,25). Indeed, with the use of human cell lines, it has been shown that NRP1 enhances infection by a clinical SARS-CoV-2 isolate and lentiviral particles pseudotyped with the S protein of SARS-CoV-2 by binding to this CendR motif of the furin-cleaved SARS-CoV-2 S1 protein. This NRP1-mediated increase in SARS-CoV-2 infectivity appears to be due to increased viral entry into host cells, rather than increased viral binding to the cell membrane, whilst it is amplified when ACE2 and TMPRSS2 are present. On the other hand, the rate of NRP1-enhanced SARS-CoV-2 infection in human cell cultures has been shown to be suppressed by using a monoclonal blocking antibody against the extracellular NRP1 b1b2 space, or a specific NRP1 mutant which ties the CendR-binding b1 domain (24,26,27). In addition,

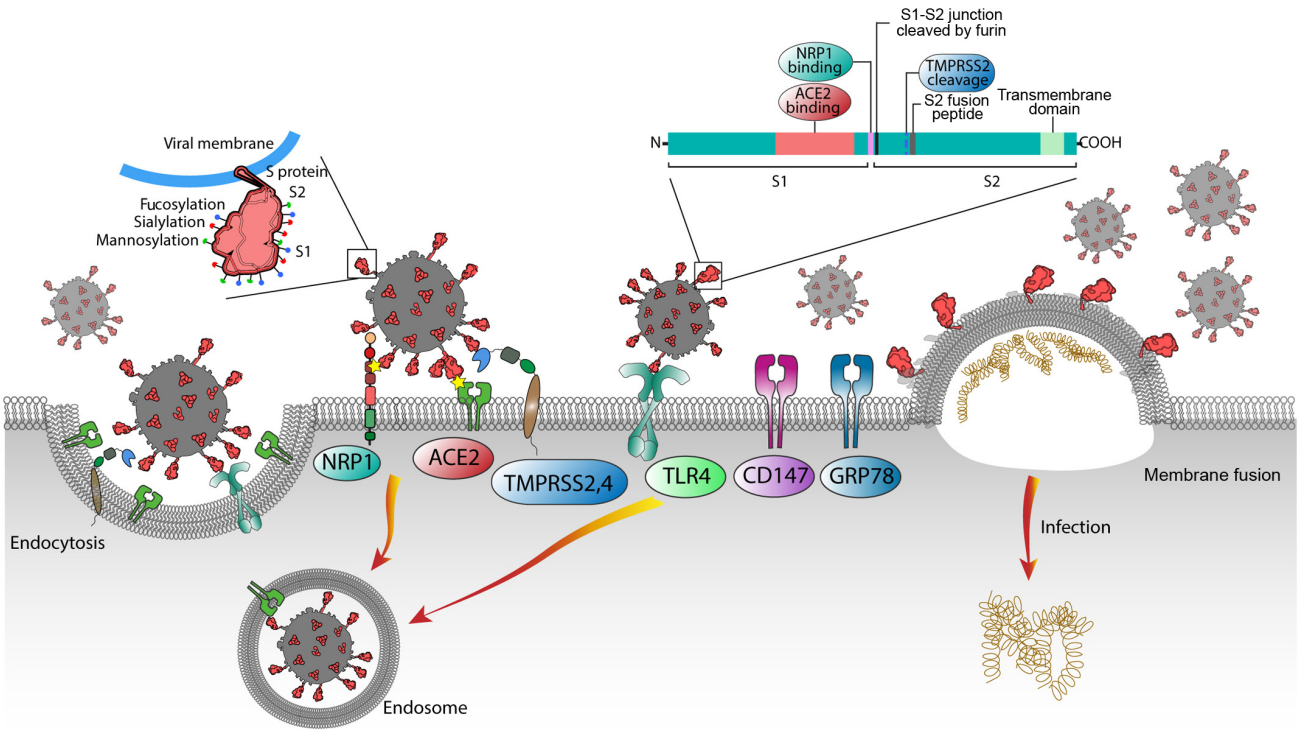

Figure 1. Schematic diagram of the known pathways facilitating the entry of SARS-CoV-2 into host cells. The SARS-CoV-2 spike (S) glycoproteins are instrumental for this process; S1 (being the cell connecting head of the molecule) binds to the ACE2 receptor, while S2 mediates the viral-cell membrane fusion by being exposed to TMPRSS2 and TMPRSS4 or other host cell proteases, such as furin, that is cleaved at the S1-S2 junction and allows the binding of S1 to NRP1. Additional molecules, including TLR4, CD147 and GRP78, have also been proposed as potential SARS-CoV-2 cell entry mediators. SARS-CoV2, severe acute respiratory syndrome coronavirus 2; ACE2, angiotensin converting enzyme 2; NPR1, neuropilin-1; TMPRSS2 and 4, transmembrane protease serine 2 and 4; TLR4, Toll-like receptor 4; GRP78, glucose-regulated protein 78; CD147, basigin (BSG).

as previously demonstrated, mutated variants of SARS-CoV-2 with a modified furin cleavage site of the S protein (erased poly-basic cleavage site or impervious to furin-intervened cleavage) were not subject to NRP1-mediated infectivity, and similarly transformations in the NRP1 b1 domain likewise restrained the NRP1-S1 interaction (24,26,27).

Notably, the upregulation of NRP1 gene expression has also been demonstrated in lung tissue samples and infected olfactory epithelial cells from patients with COVID-19; further co-staining revealed the infection of cells that were positive for oligodendrocyte transcription factor 2, which is primarily expressed by olfactory neuronal progenitors (27).

Given these data on NRP1 and since extra-pulmonary symptoms (e.g., gastrointestinal and neurological symptoms) are frequently identified in relation to COVID-19, extensive additional research is still required to identify the association between SARS-CoV-2 tropism and the mechanisms through which host cell infection mediators, such as NRP1, are dispersed across the various tissues/organs of the human body. This may further aid in the development of novel antiviral drugs against COVID-19, which for example could be targeting NRP1 and its role in promoting the entry of SARS-CoV-2 into host cells.

4. Emerging SARS-CoV-2 cell entry mediators

Following the initial findings regarding the role of host cell receptors/mediators, such as ACE2 and NRP1, data are also emerging for a number of additional cellular factors which may also facilitate or inhibit the infectivity of SARS-CoV-2, including glucose-regulated protein 78 (GRP78), basigin (BSG; CD147), kidney injury molecule-1 (KIM1), heparan sulfate (HS), ADAM metallopeptidase domain 17 [also termed tumour necrosis factor-α convertase (ADAM17)], surfactant protein D (SP-D), metabotropic glutamate receptor subtype 2 (mGluR2) and Toll-like receptor 4 (TLR4).

In this context, heat shock protein A5 (HSPA5), also termed GRP78, has been reported to be implicated in a possible route for SARS-CoV-2 cell attachment and entry. Indeed, it has been proposed that the recognition site for GRP78 is found in SARS-CoV-2 (28), and that the binding is more favourable between regions III (C391-C525) and IV (C480-C488) of the SARS-CoV-2 protein spike model and GRP78 (29). Moreover, CD147 (also known as EMMPRIN or BSG; a transmembrane glycoprotein that belongs to the large immunoglobulin super-family) has also been proposed as another molecule that can facilitate the entry of SARS-CoV-2 into host cells by endocytosis. This is supported by the findings of an *in vitro* study in which SARS-CoV-2 amplification was inhibited when CD147 was blocked in Vero E6 and BEAS-2B cell lines (30). However, based on the findings of a later study, another group argued that there was no evidence for a direct interaction between the viral spike protein and this particular molecule (31). Such contradictory findings highlight the current need for further research into the entire spectrum of potential host-pathogen interactions for SARS-CoV-2.

Furthermore, given that kidney-related complications are common in patients with COVID-19 (32), the involvement of KIM1 has been recently investigated. Accordingly, through a

series of experiments, it was previously demonstrated that KIM1 is capable of interacting with the receptor-binding domain of this new coronavirus, subsequently enabling its attachment to the plasma membrane (33). These findings suggest that this molecule may mediate and exacerbate SARS-CoV-2 infection of the kidneys (33). Moreover, KIM1 may function as a biomarker of acute kidney injury, which has been associated with a poor prognosis of patients with COVID-19 (34).

mGluR2 (GRM2), a G protein-coupled receptor which inhibits adenylyl cyclase and regulates the mechanisms through which glutamate can control cell excitability, is another potential cell entry receptor for SARS-CoV-2. Indeed, recent experiments have demonstrated that mGluR2 interacts directly with the S protein of SARS-CoV-2, whilst the knockdown of mGluR2, even though it does not affect binding to the plasma membrane, reduces the clathrin-mediated endocytosis of this virus (35). Moreover, *in vivo* studies corroborated these findings, where viral infectivity in lungs of mice was significantly reduced, when mGluR2 was knocked out (35). Of note, angiotensin II receptor type 2 is another G protein-coupled receptor, which, based on an *in silico* simulation data has revealed an interaction with the S protein of SARS-CoV-2 (36). However, further *in vitro* and/or *in vivo* studies are required to validate these initial modelling data.

Apart from direct cell entry mediators of SARS-CoV-2 into host cells, there have been molecules which appear to function as co-factors in this process, such as HS and ADAM17. Of note, HS is a co-factor for ACE2-mediated viral entry and a potential therapeutic target (e.g., using mitoxantrone) (37). On the other hand, ADAM17 has been shown to regulate the shedding of the ACE2 ectodomain (38). This is in line with its role in releasing ectodomains of a wide repertoire of cytokines, enzymes or other molecules (38,39). However, due to a potential redundancy in viral cell entry, it has been demonstrated that TMPRSS2 can compete with ADAM17 for ACE2 processing (40). Furthermore, SP-D is an innate immune molecule with a main role in clearing pathogens and apoptotic/necrotic cells from pulmonary and extra-pulmonary mucosal sites (41). Given that human SP-D can recognise the spike glycoprotein of SARS-CoV, a recent study demonstrated that the treatment of 293T cells overexpressing ACE2 with a recombinant fragment of human SP-D (rfhSP-D) inhibited the interaction of the S1 spike proteins with the cell membrane, raising the possibility of an alternative therapeutic target (42). Indeed, in another study, when clinical samples were used, treatment with rfhSP-D inhibited viral replication, and appeared to be more efficient than the antiviral drug remdesivir, when Vero cells were treated *in vitro* (43). Currently, a Phase 1 clinical trial (ClinicalTrials.gov identifier: NCT04659122) is ongoing, entitled: 'Phase 1b Open-label, Single Arm, Cohort Dose Escalation Study Evaluating the Safety, Tolerability, and Feasibility of Intervention With AT-100 (rhSP-D) in Intubated Patients Receiving Invasive Mechanical Ventilation With Severe COVID-19 Infection'. Finally, in a previous observational study on 39 patients with COVID-19, SP-D levels were found to be significantly higher in severe compared to mild cases, suggesting the possibility of its use as a biomarker of severity as well (44).

Finally, another innate immune molecule that has gained increasing interest in the context of COVID-19 is TLR4, which represents a key cell surface receptor that induces the secretion of pro-inflammatory cytokines and interferons to fight infection (45). Indeed, there is accumulating evidence to indicate that SARS-CoV-2 can bind to TLR4, whilst treatment with resatorvid (a TLR4 specific inhibitor) has been shown to abolish the secretion of IL1B by SARS-CoV-2 in THP-1 cells (46,47).

Overall, identifying the underlying mechanisms that facilitate the entry of SARS-CoV-2 into host cells (Fig. 1) is currently the focus of intensive research which is expected to provide further valuable insight into the pathophysiology of COVID-19, identifying the human tissues/organs that are more vulnerable to a SARS-CoV-2 infection. Ultimately, this may also aid in the development of more precise treatments against COVID-19 sequelae. A list of the 'canonical' and potential cell entry mediators is provided in Table I.

5. Identification of miRNAs that can target and regulate the gene expression of SARS-CoV-2 cell entry mediators

miRNAs are single-strand, endogenous, non-coding, short RNAs of 22-25 nucleotides in length, which can regulate gene expression post-transcriptionally and subsequently alter signalling pathways (53). A series of review articles have elegantly summarised the functions of miRNAs in health and disease (54-57). Of note, miRNAs regulate the expression of key inflammatory cytokines involved in the massive recruitment of immune cells to the lungs, such as IL6 and TNFα. Several studies have demonstrated that changes in the expression of miRNAs in patients with COVID-19 may be a predictor of tissue damage and lung inflammation, whilst miRNAs can also be potent diagnostic biomarkers for COVID-19 (58-60).

Several miRNAs have been either predicted or shown to target SARS-CoV-2 RNAs (61). Indeed, a number of miRNAs have been found to target the RNA S glycoprotein sequence of SARS-CoV-2, which interacts with ACE2 for viral entry into host cells, including hsa-miR-4661-3p (62), hsa-miR-510-3p, hsa-miR-624-5p, hsa-miR-497-5p (63), hsa-miR-622, hsa-miR-761, miR-A3r, hsa-miR-15b-5p, miR-A2r, hsa-miR-196a-5p (64), and miR-338-3p, miR-4661-3p, miR-4761-5p, hsa-miR-4464, hsa-miR-1234-3p, hsa-miR-7107-5p and hsa-miR-885-5p, which have been shown to bind to the receptor binding domain of the S gene (62).

As such, a number of studies have identified host cell miRNAs that target different SARS-CoV-2 proteins. Considering the aforementioned significance of ACE2 and TMPRSS2 in SARS-CoV-2 infection, in a previous study, a TargetScan analysis revealed several miRNAs that could directly target these two receptors, including hsa-mi-200b-3p, hsa-miR-200c-3p and hsa-miR-429 for ACE2, and hsa-let7a-5p, hsa-let7b-5p, hsa-let7c-5p, hsa-let7d-5p, hsa-let7e-5p, hsa-let7f-5p, hsa-let7g-5p, hsa-let7i-5p, hsa-miR-98-5p, has-miR-4458 and hsa-miR-4500 for TMPRSS2; thus, these miRNAs may be utilised as potent therapeutic molecules to regulate key proteins that are required for viral entry to the host airway/lung epithelial cells (59). Moreover, TMPRSS2, which activates the spike protein of SARS-CoV-2 to promote viral infection, has been predicted to be regulated by MR147-3p in the gut (62).

Overall, several cell host miRNAs that target the ACE2 and TMPRSS2 genes can prevent attachment and the entry of SARS-CoV-2 into different tissues. For example, hsa-miR-98-5p has been shown to directly target the 3'-UTR of TMPRSS2, both in human lung microvascular endothelial cells (HMVEC-L) and human umbilical vein

Table I. Genes related to SARS-CoV-2 entry into host cells.

Cell entry mediators	Abbreviation	Proposed action	(Refs.)
Angiotensin converting enzyme 2	ACE2	Canonical receptor for the S glycoprotein (S1 domain)	(4,48,49)
Transmembrane protease, serine 2 and serine 4	TMPRSS2 TMPRSS4	Mediate fusion of viral and host cell membranes after proteolytic cleavage at the S1/S2 region	(7,19,50,51)
Neuropilin-1	NRP1	Additional SARS-CoV-2 host cell receptor, increasing its infectivity and contributing to its tropism	(20,23-25,48)
Toll-like receptor 4	TLR4	Potential alternative receptor for SARS-CoV-2	(46,47,52)
Kidney injury molecule-1	KIM1	Potential interaction with SARS-CoV-2 receptor-binding domain	(33,34)
Basigin	BSG/CD147	Potential facilitator of the entry of SARS-CoV-2 into host cells by endocytosis	(30,31)
Heat shock protein A5 or glucose regulating protein 78	HSPA5/GRP78	Possible route for SARS-CoV-2 cell attachment and entry	(28,29)
ADAM metallopeptidase domain 17 or TNF-α convertase enzyme	ADAM17	Regulates shedding of the ACE2 ectodomain	(10,38,39)

SARS-CoV2, severe acute respiratory syndrome coronavirus 2.

Table II. List of miRNAs that indicate strong binding potential against transmembrane serine protease 4.

Target rank	Target score	miRNA name	miRNA sequence
1	88	hsa-miR-6721-5p	5'-ugggcaggggcuuauuguaggag-3'
2	85	hsa-miR-3671	5'-aucaaauaaggacuagucugca-3'
3	82	hsa-miR-496	5'-ugaguauuacauggccaaucuc-3'
4	81	hsa-miR-551b-3p	5'-gcgacccauacuugguuucag-3'
5	81	hsa-miR-551a	5'-gcgacccacucuugguuucca-3'

endothelial cells (65). Furthermore, the hsa-let-7e/hsa-mir-125a and hsa-mir-141/hsa-miR-200 miRNA families inhibit ACE2 and TMPRSS2 expression (66). It has also been shown that miR-200c is essential for SARS-CoV-2 binding to the ACE2 receptor and entry into cardiomyocytes (67), while miR-98-5p can inhibit TMPRSS2 expression in human endothelial cells (65). In a previous study, bioinformatics analysis revealed that lysine-specific demethylase 5B can regulate ACE2 and TMPRSS2 via the transcriptional repression of let-7e/miR-125a and miR-141/miR-200 miRNAs; thus, these miRNAs are crucial for the function of ACE2 and TMPRSS2 (66).

Notably, a recent study provided novel evidence of an interplay between host miRNAs and SARS-CoV-2, as well as the mechanisms through which these interactions can drive pathogenesis by dysregulating certain immune pathways (68). A recent mini-review, provided an excellent overview of the role of COVID-19-associated miRNAs as potential therapeutic targets and biomarkers (69). Currently, there are a number of clinical trials/studies investigating miRNAs in the context of COVID-19. For example, in an observational study on the expression of cytokines, transcriptome and miRNAs in COVID-19 patients is currently recruiting (ClinicalTrials.gov identifier: NCT04583566). Similarly, 'The Effect of miRNA and Epigenetic Modifications on Prognosis in Covid-19 Infection' will also be investigated (ClinicalTrials.gov Identifier: NCT04411563), whilst another study aims to assess the mechanisms through which miRNA levels relate to viral infection (ClinicalTrials.gov Identifier: NCT04346160). In terms of therapeutics, a phase 2 trial is currently enrolling by invitation, with miRNA-containing exosomes as an aerosol inhaler (ClinicalTrials.gov Identifier: NCT04602442).

Of note, the targeting of PARP-1 by miRNAs has been suggested to hold therapeutic value for COVID-19-related pathologies (70). In a recent *in silico* study, a number of hypothalamic miRNAs that can bind and regulate ACE2 and TMPRSS2 were identified (71). The authors of that study concluded that these miRNAs may be used in the search for novel therapies for the neurological symptoms in patients with COVID-19. The present study expanded on these initial observations by identifying potential miRNA modulators of all remaining SARS-CoV-2 cell entry mediators, using miRDB, an online database for miRNA target prediction and functional annotations (mirdb.org) (72,73). For the present study, the target prediction score was set >80 for higher confidence in the interactions. As such, Table II provides a list of five potential candidate miRNAs against TMPRRS4; Table III provides a list of 69 miRNAs for NRP1; Table IV indicates 36 potential miRNA interactions with ADAM17; Table V presents five interactions with KIM1; Table VI presents a list of 27 interactions with GRP78 (HSPA5); Table VII presents

Table III. List of miRNAs that indicate strong binding potential against neuropilin-1.

Target rank	Target score	miRNA name	miRNA sequence
1	98	hsa-miR-3646	5'-aaaaugaaaugagcccagccca-3'
2	97	hsa-miR-7977	5'-uucccagccaacgcacca-3'
3	96	hsa-miR-6844	5'-uucuuuguuuuuaauucacag-3'
4	95	hsa-miR-124-3p	5'-uaaggcacgcggugaaugccaa-3'
5	95	hsa-miR-3133	5'-uaaagaacucuuaaaacccaau-3'
6	95	hsa-miR-5094	5'-aaucagugaaugccuugaaccu-3'
7	95	hsa-miR-506-3p	5'-uaaggcacccuucugaguaga-3'
8	95	hsa-miR-153-5p	5'-ucauuuuugugauguugcagcu-3'
9	94	hsa-miR-4755-5p	5'-uuucccuucagagccuggcuuu-3'
10	94	hsa-miR-148a-3p	5'-ucagugcacuacagaacuuugu-3'
11	94	hsa-miR-152-3p	5'-ucagugcaugacagaacuugg-3'
12	94	hsa-miR-148b-3p	5'-ucagugcaucacagaacuuugu-3'
13	94	hsa-miR-3148	5'-uggaaaaaacuggugugugcuu-3'
14	94	hsa-miR-5006-3p	5'-uuucccuuuccauccuggcag-3'
15	93	hsa-miR-1285-3p	5'-ucugggcaacaaagugagaccu-3'
16	93	hsa-miR-548an	5'-aaaaggcauugugguuuuug-3'
17	93	hsa-miR-3686	5'-aucuguaagagaaaguaaauga-3'
18	93	hsa-miR-651-3p	5'-aaaggaaaguguauccuaaaag-3'
19	93	hsa-miR-5189-5p	5'-ucugggcacaggcggauggacagg-3'
20	92	hsa-let-7a-3p	5'-cuauacaaucuacugucuuuc-3'
21	92	hsa-miR-98-3p	5'-cuauacaacuuacuacuuuccc-3'
22	92	hsa-miR-6860	5'-acugggcagggcuguggugagu-3'
23	92	hsa-miR-1-3p	5'-uggaauguaaagaaguauguau-3'
24	92	hsa-miR-612	5'-gcugggcagggcuucugagcuccuu-3'
25	92	hsa-miR-3187-5p	5'-ccugggcagcguguggcugaagg-3'
26	92	hsa-let-7b-3p	5'-cuauacaaccuacugccuuccc-3'
27	92	hsa-miR-206	5'-uggaauguaaggaaguguguggg-3'
28	92	hsa-miR-5004-5p	5'-ugaggacagggcaaauucacga-3'
29	92	hsa-let-7f-1-3p	5'-cuauacaaucuauugccuuccc-3'
30	91	hsa-miR-510-3p	5'-auugaaaccucuaagagugga-3'
31	90	hsa-miR-320d	5'-aaaagcuggguugagagga-3'
32	90	hsa-miR-613	5'-aggaauguuccuucuuugcc-3'
33	90	hsa-miR-4429	5'-aaaagcugggcugagaggcg-3'
34	90	hsa-miR-320c	5'-aaaagcuggguugagagggu-3'
35	90	hsa-miR-6806-3p	5'-ugaagcucugacauuccugcag-3'
36	90	hsa-miR-3928-5p	5'-ugaagcucuaagguuccgccugc-3'
37	90	hsa-miR-320a-3p	5'-aaaagcuggguugagagggcga-3'
38	90	hsa-miR-338-3p	5'-uccagcaucagugauuuuguug-3'
39	90	hsa-miR-9-5p	5'-ucuuugguuaucuagcuguauga-3'
40	90	hsa-miR-1322	5'-gaugaugcugcugaugcug-3'
41	90	hsa-miR-320b	5'-aaaagcuggguugagagggcaa-3'
42	89	hsa-miR-150-3p	5'-cugguacaggccugggggacag-3'
43	89	hsa-miR-4261	5'-aggaaacagggaccca-3'
44	88	hsa-miR-6733-3p	5'-ucagugucuggauuuccuag-3'
45	88	hsa-miR-5701	5'-uuauugucacguucugauu-3'
46	87	hsa-miR-3920	5'-acugauuaucuuaacucucuga-3'
47	87	hsa-miR-147b-5p	5'-uggaaacauuucugcacaaacu-3'
48	87	hsa-miR-4724-5p	5'-aacugaaccaggagugagcuucg-3'
49	87	hsa-miR-4251	5'-ccugagaaaagggccaa-3'
50	86	hsa-miR-137-3p	5'-uuauugcuuaagaauacgcguag-3'
51	86	hsa-miR-4789-5p	5'-guauacaccugauauguguaug-3'
52	86	hsa-miR-570-3p	5'-cgaaaacagcaauuaccuuugc-3'

Table III. Continued.

Target rank	Target score	miRNA name	miRNA sequence
53	85	hsa-miR-4801	5'-uacacaagaaaaccaaggcuca-3'
54	84	hsa-miR-24-3p	5'-uggcucaguucagcaggaacag-3'
55	84	hsa-miR-587	5'-uuuccauaggugaugagucac-3'
56	83	hsa-miR-4324	5'-cccugagacccuaaccuuaa-3'
57	83	hsa-miR-181a-2-3p	5'-accacugaccguugacuguacc-3'
58	82	hsa-miR-186-5p	5'-caaagaauucuccuuuuggggcu-3'
59	82	hsa-let-7f-2-3p	5'-cuauacagucuacugucuuucc-3'
60	82	hsa-miR-6843-3p	5'-auggucuccuguucucugcag-3'
61	82	hsa-miR-1185-1-3p	5'-auauacaggggagacucuuau-3'
62	82	hsa-miR-6848-3p	5'-guggucucuuggccccccag-3'
63	82	hsa-miR-6853-3p	5'-uguucauuggaacccugcgcag-3'
64	82	hsa-miR-1185-2-3p	5'-auauacaggggagacucucau-3'
65	81	hsa-miR-6736-3p	5'-ucagcuccucucuacccacag-3'
66	81	hsa-miR-4277	5'-gcaguucugagcacaguacac-3'
67	81	hsa-miR-3662	5'-gaaaaugaugaguagugacugaug-3'
68	81	hsa-miR-1289	5'-uggaguccaggaaucugcauuuu-3'
69	81	hsa-miR-1825	5'-uccagugcccuccucucc-3'

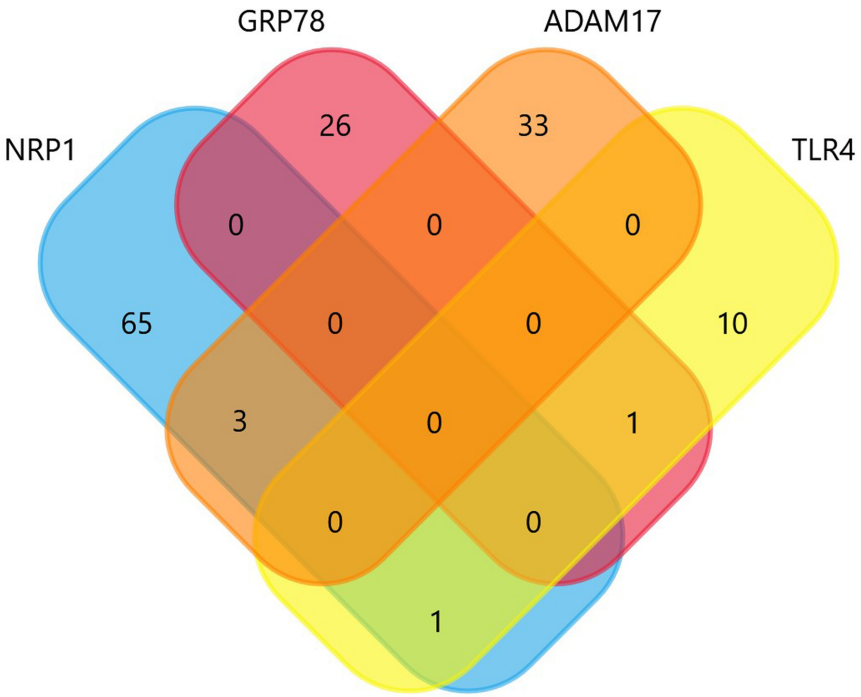

Figure 2. Venn diagram describing shared miRNAs between NRP1, GRP78, ADAM17 and TLR4. NRP1 and ADAM17 share three common miRNAs: hsa-miR-148a-3p, hsa-miR-152-3p and hsa-miR-148b-3p. TLR4 and NRP1 share one common miRNA, hsa-miR-587; whereas TLR4 and GRP78 share hsa-miR-338-5p as a common miRNA.

only one possible interaction identified between a miRNA and CD147 (BSG); Table VIII presents 12 interactions identified between miRNAs and TLR4; and finally, Table IX outlines five possible interactions of these regulatory RNAs with mGluR2.

Further analysis using Venn diagrams (Fig. 2), revealed that there were three common miRNAs amongst NRP1 and ADAM17, namely hsa-miR-148a-3p, hsa-miR-152-3p and hsa-miR-148b-3p. One common miRNA, i.e., hsa-miR-587, was also shared between TLR4 and NRP1, and another miRNA (hsa-miR-338-5p) between TLR4 and GRP78. Of note, hsa-miR-148a-3p was also shared with ACE2, as previously reported (71). Moreover, hsa-miR-587 was recently identified as a top miRNA regulating ACE2 networks; it was

Table IV. List of miRNAs that indicate strong binding potential against ADAM metallopeptidase domain 17.

Target rank	Target score	miRNA name	miRNA sequence
1	99	hsa-miR-3163	5'-uauaaaaugagggcaguaagac-3'
2	97	hsa-miR-548ah-3p	5'-caaaaacugcaguuacuuuugc-3'
3	97	hsa-miR-548am-3p	5'-caaaaacugcaguuacuuuugu-3'
4	97	hsa-miR-548x-3p	5'-uaaaaacugcaauuacuuuc-3'
5	97	hsa-miR-4465	5'-cucaaguagucugaccagggga-3'
6	97	hsa-miR-548aj-3p	5'-uaaaaacugcaauuacuuuua-3'
7	96	hsa-miR-548ae-3p	5'-caaaaacugcaauuacuuuca-3'
8	96	hsa-miR-548aq-3p	5'-caaaaacugcaauuacuuuugc-3'
9	96	hsa-miR-1297	5'-uucaaguaauucaggug-3'
10	96	hsa-miR-26a-5p	5'-uucaaguaauccaggauaggcu-3'
11	96	hsa-miR-26b-5p	5'-uucaaguaauucaggauaggu-3'
12	96	hsa-miR-548j-3p	5'-caaaaacugcauuacuuuugc-3'
13	89	hsa-miR-507	5'-uuuugcaccuuuuggagugaa-3'
14	88	hsa-miR-5697	5'-ucaaguaguuucaugauaaagg-3'
15	88	hsa-miR-4762-5p	5'-ccaaaucuugaucagaagccu-3'
16	88	hsa-miR-4719	5'-ucacaaaucuauaauaugcagg-3'
17	88	hsa-miR-5197-5p	5'-caauggcacaaacucauucuuga-3'
18	88	hsa-miR-4686	5'-uaucugcugggcuuucugguguu-3'
19	87	hsa-miR-95-5p	5'-ucaauaaaugucuguugaauu-3'
20	87	hsa-miR-8064	5'-agcacacugagcgagcggac-3'
21	86	hsa-miR-7109-3p	5'-caagccucuccugcccuuccag-3'
22	86	hsa-miR-513a-5p	5'-uucacagggaggugucau-3'
23	84	hsa-miR-5195-3p	5'-auccaguucucugaggggcu-3'
24	84	hsa-miR-557	5'-guuugcacgggugggccuugucu-3'
25	84	hsa-miR-145-5p	5'-guccaguuuucccaggaaucccu-3'
26	84	hsa-miR-12136	5'-gaaaaagucauggaggcc-3'
27	84	hsa-miR-6126	5'-gugaaggcccggcggaga-3'
28	83	hsa-miR-4799-5p	5'-aucuaaaugcagcaugccaguc-3'
29	83	hsa-miR-148a-3p	5'-ucagugcacuacagaacuuugu-3'
30	83	hsa-miR-152-3p	5'-ucagugcaugacagaacuugg-3'
31	83	hsa-miR-4464	5'-aagguuuggauagaugcaaua-3'
32	83	hsa-miR-4748	5'-gaggguuuggggaggauuugcu-3'
33	83	hsa-miR-605-3p	5'-agaaggcacuaugagauuuaga-3'
34	83	hsa-miR-148b-3p	5'-ucagugcaucacagaacuuugu-3'
35	83	hsa-miR-767-5p	5'-ugcaccaugguugucugagcaug-3'
36	81	hsa-miR-548l	5'-aaaaguauuugcgggguuuuguc-3'

thus concluded that these miRNAs can be used in the personalized diagnosis of patients with COVID-19 (74).

Moreover, making use of the miRNA atlas (ccb-web.cs.uni-saarland.de/tissueatlas), it was evident that the miRNAs listed in Tables I-VIII have a wide distribution in the human body and co-localise with SARS-CoV-2 cell entry mediators (Fig. 3). For example, hsa-miR-152-3p is primarily expressed in the epididymis, colon, artery, oesophagus, thyroid, whereas hsa-miR-338-5p is mostly present in the kidneys, brain and spleen. This co-localisation provides a higher order of complexity on the mechanisms through which these mediators may be regulated by miRNAs. Future studies are required to evaluate their therapeutic or diagnostic potential.

6. Conclusion

The pathophysiology of COVID-19 is mainly dependent on the underlying mechanisms that mediate the entry of SARS-CoV-2 into the host cells of the various human tissues/organs. Based on the growing body of evidence regarding these mechanisms, it is evident that there is an array of factors/mediators which play crucial roles in these processes, often in synergistic/complimentary ways. The complexity of these viral-host interactions appears to be further increased when the co-localization of SARS-CoV-2 host cell entry mediators in different human tissues/organs is considered, as well as when considering the potential modulatory effects that several miRNAs can have on

Table V. List of miRNAs that indicate strong binding potential against hepatitis A virus cellular receptor 1 (HAVCR1, KIM1).

Target rank	Target score	miRNA name	miRNA sequence
1	90	hsa-miR-4255	5'-caguguucagagaugga-3'
2	87	hsa-miR-4738-3p	5'-ugaaacuggagcgccuggagga-3'
3	86	hsa-miR-3117-3p	5'-auaggacucauauagugccag-3'
4	85	hsa-miR-3171	5'-agauguauggaaucuguauauauc-3'
5	83	hsa-miR-383-5p	5'-agaucagaaggugauuguggcu-3'

Table VI. List of miRNAs that indicate strong binding potential against heat shock protein family A (Hsp70) member 5 (HSPA5 or GRP78).

Target rank	Target score	miRNA name	miRNA sequence
1	99	hsa-miR-3121-3p	5'-uaaauagaguaggcaaaggaca-3'
2	97	hsa-miR-5688	5'-uaacaaacaccuguaaaacagc-3'
3	96	hsa-miR-635	5'-acuugggcacugaaacaaugucc-3'
4	96	hsa-miR-6774-5p	5'-acuugggcaggagggacccuguaug-3'
5	95	hsa-miR-495-3p	5'-aaacaaacauggugcacuucuu-3'
6	93	hsa-miR-338-5p	5'-aacaauauccuggugcugagug-3'
7	91	hsa-miR-7-2-3p	5'-caacaaaucccagucuaccuaa-3'
8	91	hsa-miR-7-1-3p	5'-caacaaaucacagucugccaua-3'
9	90	hsa-miR-30d-3p	5'-cuuucagucagauguuugcugc-3'
10	89	hsa-miR-199a-5p	5'-cccaguguucagacuaccuguuc-3'
11	89	hsa-miR-199b-5p	5'-cccaguguuuagacuaucuguuc-3'
12	89	hsa-miR-4699-3p	5'-aauuuacucugcaaucuucucc-3'
13	88	hsa-miR-7854-3p	5'-ugaggugaccgcagaugggaa-3'
14	87	hsa-miR-4650-3p	5'-agguagaaugaggccugacau-3'
15	85	hsa-miR-222-5p	5'-cucaguagccaguguagauccu-3'
16	84	hsa-miR-16-1-3p	5'-ccaguauuaacugugcugcuga-3'
17	84	hsa-miR-30e-3p	5'-cuuucagucggauguuuacagc-3'
18	84	hsa-miR-148a-5p	5'-aaaguucugagacacuccgacu-3'
19	84	hsa-miR-30a-3p	5'-cuuucagucggauguuugcagc-3'
20	84	hsa-miR-4451	5'-ugguagagcugaggaca-3'
21	84	hsa-miR-7162-3p	5'-ucugagguggaacagcagc-3'
22	83	hsa-miR-6777-3p	5'-uccacucuccuggcccccag-3'
23	82	hsa-miR-770-5p	5'-uccaguaccacgugucagggcca-3'
24	82	hsa-miR-4712-5p	5'-uccaguacaggucucucauuuc-3'
25	81	hsa-miR-3606-3p	5'-aaaauuucuuucacuacuuag-3'
26	81	hsa-miR-513c-3p	5'-uaaauuucaccuuucugagaaga-3'
27	81	hsa-miR-513a-3p	5'-uaaauuucaccuuucugagaagg-3'

Table VII. One miRNA which exhibits strong binding potential against basigin (BSG, CD147).

Target rank	Target score	miRNA name	miRNA sequence
1	93	hsa-miR-1252-5p	5'-agaaggaaauugaauucauuua-3'

the local expression of these mediators. Accordingly, intensive research is still warranted in this field in order to precisely characterise these underlying mechanisms, with the ultimate objective of aiding in the development of novel treatments, which will act by blocking the entry of SARS-CoV-2 into susceptible human host cells.

Table VIII. List of miRNAs that indicate strong binding potential against Toll-like receptor 4.

Target rank	Target score	miRNA name	miRNA sequence
1	93	hsa-miR-448	5'-uugcauauguaggaugucccau-3'
2	91	hsa-miR-3924	5'-auauguauaugugacugcuacu-3'
3	89	hsa-miR-627-3p	5'-ucuuuucuuugagacucacu-3'
4	88	hsa-miR-338-5p	5'-aacaauauccuggugcugagug-3'
5	88	hsa-miR-4272	5'-cauucaacuagugauugu-3'
6	87	hsa-miR-10397-5p	5'-uccuugaccugaugcuguaggg-3'
7	87	hsa-miR-5583-3p	5'-gaauaugggguauauuaguuugg-3'
8	85	hsa-miR-4760-3p	5'-aaauucauguucaaucuaaacc-3'
9	85	hsa-miR-4652-3p	5'-guucuguuaacccaucccccuca-3'
10	84	hsa-miR-1243	5'-aacuggaucaauuauaggagug-3'
11	81	hsa-miR-587	5'-uuuccauaggugaugagucac-3'
12	81	hsa-miR-4678	5'-aagguauuguucagacuuauga-3'

Table IX. List of miRNAs that indicate strong binding potential against metabotropic glutamate receptor subtype 2.

Target rank	Target score	miRNA name	miRNA sequence
1	86	hsa-miR-4492	5'-ggggcugggcgcgcgcc-3'
2	84	hsa-miR-555	5'-aggguaagcugaaccucugau-3'
3	83	hsa-miR-4436b-3p	5'-cagggcaggaagaaguggacaa-3'
4	83	hsa-miR-497-3p	5'-caaaccacacugugguguuaga-3'
5	82	hsa-miR-4498	5'-ugggcuggcagggcaagugcug-3'

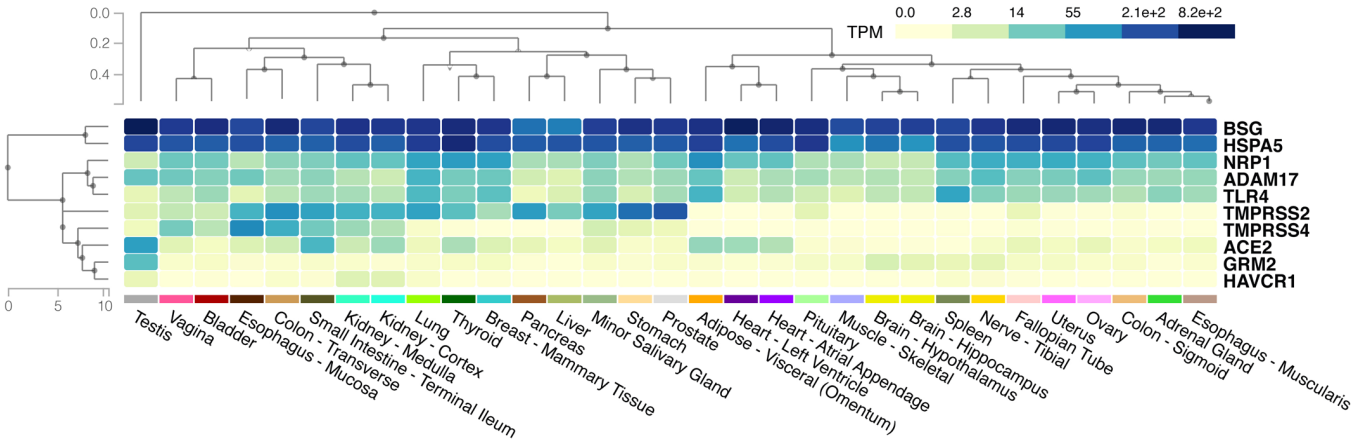

Figure 3. Distribution and co-localisation of all potential cell entry mediators. BSG, basigin (CD147); HSPA5, heat shock protein A5; ADAM17, ADAM metallopeptidase domain 17 (also termed tumour necrosis factor-α convertase); TMPRSS2 and 4, transmembrane protease serine 2 and 4; ACE2, angiotensin converting enzyme 2; GRM2, glutamate receptor subtype 2 (mGluR2); HAVCR1, hepatitis A virus cellular receptor 1 (also termed kidney injury molecule-1, KIM1).

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Availability of data and materials

The data used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

PK, EK, SS, HSR and IK contributed to the conceptualization, methodology, data curation, investigation, visualization, drafting, editing and reviewing of the manuscript. HSR, DAS and SS contributed to the literature search, and the drafting and critical revision of the manuscript. All authors have read and approved the final manuscript. SS and EK confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

DAS is the Editor-in-Chief for the journal, but had no personal involvement in the reviewing process, or any influence in terms of adjudicating on the final decision, for this article. The other authors declare that they have no competing interests.

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