APPLICATION NOTE

Infectious disease research

SARS-CoV-2 viral entry factors

Studying the molecular targets using TaqMan Assays

Introduction

Research into SARS-CoV-2 is a rapidly emerging field. This novel zoonotic coronavirus combines high transmissibility with high severity, making it critically important to understand how it works. Studies have approached the problem of SARS-CoV-2 from many directions, including investigating existing medications for utility against it [1,2], studying its relationship with its previous host species [3,4], and trying to determine its relationship to other coronaviruses [5-8]. Each provides an important piece of the overall picture, attempting to bring the virus from immediate pressing concern to lower-level threat.

An important aspect of this research is studying SARS-CoV-2's relationship to the human body, in particular the molecular targets it uses to gain entry into cells. Viruses work by entering cells and commandeering their protein synthesis and reproductive machinery to generate copies of themselves, which leads to cell death and tissue damage. Viruses usually cannot bore or sneak into cells on their own; typically they rely on proteins that are already part of cells to gain entry, manipulating or tricking the cell into bringing the viral particles inside. Called "entry factors", these proteins have a variety of functions, including maintenance of lung health and serving as components of signal transduction pathways. The entry factors a virus uses influence which tissues it attacks and have a sizable impact on how a viral infection affects its host. To understand how SARS-CoV-2 affects the human body and how to combat those effects, it is critical to identify entry factors and the variations thereof that facilitate viral entry.

Noteworthy entry factors for SARS-CoV-2 that have already been identified include ACE2, ANPEP, BSG, CLEC4G, CTSB, CTSL, DPP4, FURIN, NRP1, TMPRSS2, TMPRSS4, TMPRSS11A, and TMPRSS11B. Specific Applied Biosystems™ TaqMan® Gene Expression Assays provide a way to study each of these in detail, and the Applied Biosystems™ TaqMan® Array Coronavirus Entry Factor Panel provides a means to study them all at once.

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TaqMan Array Coronavirus Entry Factor Panel

Applied Biosystems™ TaqMan® Assay technology is the gold standard of performance, quality, and specificity in gene expression analysis by quantitative polymerase chain reaction (qPCR), optimized to work with numerous platforms and equipment setups. Predesigned TaqMan Assays exist for thousands of individual genes across dozens of species, which can be an ideal starting point for researchers with known genes of interest. For scientists looking at a large number of specific genes, such as those of an entire functional pathway, however, using single assays for individual genes is often not cost-effective. When looking at a large number of genes at once, a much easier approach is to instead identify markers of interest using a panel that targets multiple genes, allowing subsequent research to focus directly on the specific genes that the broader assay flagged as noteworthy. For SARS-CoV-2's entry factors, that means initial identification using the TaqMan Array Coronavirus Entry Factor Panel.

The TaqMan Array Coronavirus Entry Factor Panel enables researchers to study all 13 of the most important entry factors for SARS-CoV-2 in a single panel: *ACE2, ANPEP, BSG, CLEC4G, CTSB, CTSL, DPP4, FURIN, NRP1, TMPRSS2, TMPRSS4, TMPRSS11A, and TMPRSS11B*. Researchers have the option to modify the preconfigured layout to add or subtract assays to fit their needs. This panel is available in human, mouse, and rat versions and comes in varieties optimized for 0.2 mL 96-well plates, 0.1 mL 96-well plates, 384-well Applied Biosystems™ TaqMan® Array Cards, and Applied Biosystems™ OpenArray™ plates.

TaqMan Gene Expression Assays

For researchers interested in focusing on specific entry factors rather than the full set of prominent targets, TaqMan Assays provide many options. TaqMan Assays come predesigned and preoptimized for thousands of genes across dozens of species, in a wide range of formats and sizes. These options provide researchers with the flexibility needed to obtain fast, reliable, and accurate results with off-the-shelf assay kits rather than laboriously custom-designed alternatives.

Multiple TaqMan Assays exist for most gene products, including many of SARS-CoV-2's entry factors. Choosing an assay depends in part on one's specific target and research goals, with different TaqMan Assays targeting different exons and variants. Some of the ways to choose an assay are discussed here.

Get the details right

When searching for TaqMan Assays for a specific gene, one of the ways to distinguish between options is to look at the assay design details. Some example considerations follow here.

Exon targets

Most often, the difference between TaqMan Assays designed for the same gene is in the specific transcript location they target. All assays for the same gene will target products of that gene, but they will flag different sequences for amplification and quantification. Checking the list of transcripts targeted by a given assay provides important information about how the assay works and whether it is suited to a given use case. An experiment premised on a specific splice variant must include its specific RefSeq accession and leave out all others, whereas an experiment with more general needs benefits from an assay that includes more transcripts. The search tools available for TaqMan Assays allow the user to check which transcripts an assay detects, and provide detailed RefSeq and GenBank information for each transcript.

Genomic DNA

A related consideration is whether the probe spans an exon–exon junction. Probes that do not span an exon–exon junction carry the risk of amplifying residual genomic DNA, making the intermediate step of using DNase to eliminate genomic DNA much more important. TaqMan Assay search results provide the ability to observe the relationships between all exons of the target gene, the location of each exon on the gene, and which exons each available assay will target, enabling researchers to choose these attributes with fine-grained detail.

Source quality

Amplicon length presents a balancing act in many experiments. A large amplicon helps assure specificity, protecting against off-target replication, at the cost of less-than-ideal amplification efficiency. However, if an experiment involves testing preserved or degraded tissue, such as formalin-fixed, paraffin-embedded (FFPE) samples, a smaller amplicon might be necessary. In such samples, genetic material might be too degraded for a long amplicon assay to amplify with good efficiency, but a smaller amplicon assay can still amplify with the desired efficiency.

Citation density

For some research, making sure one's results are directly comparable to previous work is important. Using the third-party Bioz database, the TaqMan Assay search tool enables users to find lists of publications that used particular assays. The assay with the highest number of citations in this database is designated "Most Citations" and enables new research to be most effectively compared and considered against previous work.

Best-coverage assays

It is often not necessary to target specific transcript variants. In fact, most of the time, standard gene expression studies benefit from the "best-coverage" assay for the target gene, designed to detect the maximum number of transcripts of the gene of interest and thus provide a more complete picture of that gene's expression. An assay designated "best-coverage" most often detects the greatest number of transcript variants, but the following criteria are also taken into account:

- Does not detect gene products with similar sequences (homologs)
- Is designed across an exon–exon junction to reduce genomic DNA contamination
- Has a short amplicon, resulting in more efficient PCR
- Does not detect off-target sequences, increasing the specificity of reactions
- Does not map to multiple genes, increasing the specificity of the experiment
- Does not target the 5′ untranslated region (UTR). The 5′ UTR of transcripts can have variable sequence between transcripts

In combination, these stipulations mean that an assay will have few off-target results and many on-target results, maximizing the ability to detect changes in expression of the target gene and reducing the chance that an unusual transcript will avoid detection.

Table 1 shows the best-coverage TaqMan Assays available for the 13 best known SARS-CoV-2 entry factors.

SARS-CoV-2 entry factors: a discussion

The 13 best known entry factors for SARS-CoV-2 vary extensively in function and significance. Their heterogeneous roles in the human body attest to the number of different organs and functions that SARS-CoV-2 can attack, and to the variety of methods that may, with additional research, prove useful for combating this serious virus. This section summarizes the 13 entry factors included in the TaqMan Array Coronavirus Entry Factor Panel, going over their functions, the other conditions they affect, and the types of studies that might benefit from examining them individually or in groups.

Table 1. Best-coverage assays for known entry factors.

Target	Human	Mouse	Rat
ACE2	Hs01085333_m1	Mm01159006_m1	Rn01416293_m1
ANPEP	Hs00174265_m1	Mm00476227_m1	Rn00578763_m1
BSG	Hs00936295_m1	Mm01144228_g1	Rn00680749_g1
CLEC4G	Hs00962163_g1	Mm01212425_m1	Rn01767375_m1
CTSB	Hs00947439_m1	Mm01310506_m1	Rn00575030_m1
CTSL	Hs00964650_m1	Mm00515597_m1	Rn04341361_m1
DPP4	Hs00897386_m1	Mm00494552_m1	Rn00562910_m1
FURIN	Hs00965485_g1	Mm00440646_m1	Rn00570970_m1
NRP1	Hs00826128_m1	Mm00435379_m1	Rn00595457_m1
TMPRSS2	Hs01122322_m1	Mm00443687_m1	Rn00590459_m1
TMPRSS4	Hs01054420_m1	Mm00520486_m1	Rn06429720_s1
TMPRSS11A	Hs00699550_m1	Mm01216640_m1	Rn01417673_m1
TMPRSS11B	Hs00699332_m1	Mm00621706_m1	Rn01413641_m1

Angiotensin-converting enzyme 2 (ACE2)

Angiotensin-converting enzyme 2 (ACE2) is a transmembrane protein found in lung, artery, heart, kidney, and intestinal tissue [9,10]. Recent evidence indicates that it is also found in smooth muscle and tissues of the nervous system [11]. Its primary function is to counter the effects of angiotensin-converting enzyme (ACE) by turning angiotensin II into angiotensin (1-7), with the downstream effect of vasodilation. ACE2 also modifies a variety of other peptides, including [des-Arg9]-bradykinin, apelin, neurotensin, dynorphin A, and ghrelin, suggesting it may have a much more expansive role in general organ function than is currently obvious [12]. ACE2 binds to SARS-CoV-2's spike protein S1, which causes ACE2 to transport the virus into the cell [13]. This has made ACE2 the focus of a great deal of research, which aims to unravel how the virus binds to it and how treatments targeted at ACE2 might prevent viral infection or limit its impact. The fact that ACE2 is also the target of medications for blood pressure disorders has been especially interesting, creating the possibility that future research might reveal these medications as useful tools against SARS-CoV-2 [14].

Alanine aminopeptidase (ANPEP)

Alanine aminopeptidase has long been recognized as an entry factor for coronaviruses. Numerous coronaviruses in several species, including humans, cats, dogs, and pigs, use this protein as part of their path into cells [15,16]. This protein is primarily found in the small intestine and renal microvillar membranes, and its role within mammalian biology appears to be as part of the digestive process, completing the breakdown of proteins hydrolyzed by pancreatic and gastric secretions. Its role in the kidney is less clear but likely also related to absorption. In addition to serving as part of digestion, alanine aminopeptidase may also help process signal peptides from elsewhere in the body, and defects in ANPEP are associated with leukemia and lymphoma [17]. Deficiency of alanine aminopeptidase activity is shown to be protective against coronavirus infection in pigs [16], and the expression pattern of *ANPEP* is very similar to that of the high-priority SARS-CoV-2 target *ACE2* [18], suggesting that studying *ANPEP* and its associated enzyme may prove useful for combating human coronaviruses such as SARS-CoV-2. In particular, research suggests that alanine aminopeptidase may be part of a cofactor network with angiotensin-converting enzyme 2 and other entry factors, rather than either one being a full-fledged entry factor on its own. This tangle of networked effects requires study to illuminate, both to understand how SARS-CoV-2 and other coronaviruses work and to learn how to better protect against them.

Basigin (BSG)

Basigin, also known as CD147 and EMMPRIN, is a highly glycosylated transmembrane protein of the immunoglobulin superfamily. It regulates the expression and activity of various soluble and membrane-bound matrix metalloproteinases and is mainly expressed in cells of epithelial, myeloid, lymphoid, and neural origin [19]. The protein plays an important part in several physiological functions such as immune response regulation, nervous system function, and reproduction and development, as well as pathological processes such as cancer and viral infections. BSG has been shown to play a role in host cell invasion by SARS-CoV [20], and recently was confirmed to facilitate SARS-CoV-2 entry into cells through interaction with the viral spike protein [21]. In fact, a functional blockade of the receptor by a monoclonal antibody significantly suppressed SARS-CoV-2 replication, suggesting that BSG could potentially be an important target for drug development [21].

C-type lectin domain family 4 member G (CLEC4G)

Lectins, such as CLEC4G, have carbohydrate-recognition domains (CRDs) or sulfated glycosaminoglycan (SGAG)-binding motifs, which drive their roles in cell–cell and cell–pathogen interactions [22]. CLEC4G, also known as LSECtin (liver and lymph node sinusoidal endothelial cell C-type lectin), is a C-type lectin, which requires calcium for binding [23]. Ligands for this type II C-type lectin are still being identified. There is some evidence that CLEC4G plays a role in Alzheimer's disease, as CLEC4G was found to interact with BACE1 and reduce its ability to cleave amyloid precursor protein (APP) to Aβ peptides [24]. Of particular interest is the role CLEC4G can play in the immune system. It has been found to facilitate entry of SARS-CoV, hCoV-229E, and Ebola virus [25-28]. Interaction of CLEC4G with the SARS spike (S) protein was found to enhance infection [25]. Given the similarity between the SARS-CoV and SARS-CoV-2 spike proteins, it is predicted that CLEC4G, and more broadly the C-type lectin protein family, will act as SARS-CoV-2 receptors and facilitate virus uptake [29].

Cathepsin B (CTSB)

Cathepsin B is a cysteine protease that plays an important role in intracellular proteolysis. It is especially significant for its role in the breakdown of extracellular matrix compounds [30] and its role in neurogenesis in the brain [31], which contributes to the formation of new memories. Cathepsin B becomes elevated in association with numerous cancers and appears to enable metastatic cancer cells to feed on extracellular materials, sustaining them as they travel [32]. The most noteworthy effects of this protease are associated with various neurological conditions. Abnormal cathepsin B activity levels contribute to post-injury neuronal cell death [33], epilepsy [30], and Alzheimer's disease [34], attesting to this protein's role in maintaining normal brain health. Cathepsin B appears to contribute to SARS-CoV-2 entry into cells in the same way that cathepsin L does [35], suggesting that the many pharmaceuticals that target cathepsin B in the context of cancer, epilepsy, and other conditions may have a role to play in protecting against the impact of SARS-CoV-2 infection. In particular, the activity of cathepsin B and similar proteins may help to explain the disproportionate impact of SARS-CoV-2 on the brain function of people infected with this virus, and cathepsin B may prove to be a viable target for preventing these effects [36].

Cathepsin L (CTSL)

Cathepsin L, like other lysosomal proteases, serves as part of the intracellular mechanism for destroying old or damaged proteins. Lysosomes are involved in immunological activity as well, used to destroy viral and other pathogenic material that cells encounter, or healthy tissue in autoimmune conditions. This means that proteins that are presented by viruses to cells in uncleaved, nonfunctional states can potentially be cleaved into functional forms by the proteases meant to destroy them, including cathepsin L. Cathepsin L has been shown to cleave SARS-CoV-2's spike protein near the site that furin does [37], similarly activating the spike protein and enabling it to bind to other entry factors. Further study of how SARS-CoV-2 interacts with cathepsin L and other lysosomal proteins is likely to yield insights similar to those gained from studying furin.

Dipeptidyl peptidase 4 (DPP4)

The protein encoded by *DPP4* is a cell-surface enzyme expressed in most cell types. This enzyme is associated with immune regulation, signal transduction, and apoptosis. As a transmembrane glycoprotein, it has traits in common with other SARS-CoV-2 entry factors, and other coronaviruses, including Middle East respiratory syndrome (MERS) coronavirus, have been found to bind to it [38]. Preliminary evidence suggests that SARS-CoV-2 can use this same enzyme as an entry factor, which is consistent with other similarities between SARS-CoV-2 and previous emergent coronaviruses [39]. *DPP4* provides an especially exciting target for SARS-CoV-2 research because it has long been targeted for pharmaceutical intervention as part of diabetes treatment. Dipeptidyl peptidase 4 mediates the release of glucagon into circulation, and inhibitors of this enzyme thus reduce glucagon, reduce circulating glucose, and increase circulating insulin [40]. The resulting connection between SARS-CoV-2 and diabetes, and the possibility that drugs used to treat diabetes could be repurposed for use against SARS-CoV-2 infection, makes this gene an especially enticing target for future research.

Furin (FURIN)

A subtilisin-like peptidase, furin is coded by the *FURIN* gene and serves to cleave precursor proteins to their biologically active versions. Furin is part of the synthesis pathway for numerous vital proteins and peptides, including parathyroid hormone and proalbumin, and is expressed throughout the body. In addition to cleaving protein precursors to convert them to active proteins, furin also cleaves the toxins produced by anthrax and *Pseudomonas* bacteria [41] and the viral spike proteins of numerous viruses, including HIV, dengue, Marburg, and SARS-CoV-2 [42]. This cleavage enables the spike proteins to fully engage with cell-surface receptors and infect cells. Compatibility between spike proteins and furin is thought to be a critical part of zoonotic transmission possibilities for viruses, helping to predict which viruses can cross between species. A spike protein compatible with human furin is one of the features that distinguishes SARS-CoV-2 from its close viral relatives [43].

Neuropilin 1 (NRP1)

Neuropilin 1 is a single-pass transmembrane glycoprotein that functions as a coreceptor for class III semaphorins and for members of the vascular endothelial growth factor and transforming growth factor beta family. The N-terminus of the protein is extracellular and contains two complement-binding domains, two coagulation factor V/VIII homology domains, and a meprin domain. The complement- and coagulation factor–binding domains engage in ligand binding while the meprin domain participates in receptor dimerization. Neuropilin 1 participates in several signaling pathways involved in angiogenesis, cardiovascular development, and neuronal development [44]. The protein has previously been shown to mediate the entry of viruses such as EBV [45] owing to the furin-mediated cleavage of the virus glycoprotein that creates a consensus [R/K] XX [R/K] motif at the C-terminus, termed the "C-end rule" (CendR) motif, known to bind to neuropilin 1. Interestingly, SARS-CoV-2 has a polybasic furin-type cleavage site at the S1-S2 junction of the spike protein, a feature absent in SARS-CoV and MERS. Recently, NRP1 was confirmed as an entry factor for SARS-CoV-2 and shown to potentiate virus infectivity by binding to the S1 CendR motif generated upon furin cleavage [46,47]. NRP1 levels were also shown to be significantly upregulated in the olfactory tissues of individuals infected with SARS-CoV-2, compared to healthy controls [61]. The protein is an important host factor for SARS-CoV-2 infection and may potentially qualify as an attractive target for treatment of the disease.

Transmembrane protease serine 2 (TMPRSS2)

TMPRSS2 was, until recently, a fairly mysterious protein. Like other serine proteases, it cleaves proteins at bonds between serine and other amino acids, usually doing so as part of signal transduction cascades [48]. Despite being found in numerous tissues, its main recognized functions are in the prostate and liver. In the liver, it activates pro-hepatocyte growth factor, leading to hepatocyte proliferation, and in the prostate, it activates protease activated receptor 2 (F2RL1) and matriptase (ST14) as part of normal prostate functioning [49]. Overexpression of *TMPRSS2* and overactivation of the protease are associated with prostate cancer metastasis through the disruption of extracellular matrices [49], and are frequently observed in prostate cancer cells [50]. In the context of SARS-CoV-2, TMPRSS2 comes up most often because it and other similar proteases appear to be a necessary part of ACE2's role as a viral entry factor, priming the viral spike protein for binding to ACE2 [51,52]. TMPRSS2 is similarly implicated in the entry pathways of numerous other viruses, including Sendai virus, human metapneumovirus, and human parainfluenza [9].

Transmembrane protease serine 4 (TMPRSS4)

Like TMPRSS2, TMPRSS4 is a transmembrane serine protease involved in signal transduction cascades. Similar to its cousin, it is expressed in multiple tissues. Overexpression of TMPRSS4 has been reported in pancreatic [53], ovarian [54], thyroid [55], colorectal [56], lung [57], breast [58], cervical [59], gallbladder [60], gastric [61], and liver cancers [62], in all cases associated with facilitating the transition from epithelial to mesenchymal stages and, with it, cancer metastasis. Abnormal *TMPRSS4* activity in the lungs is additionally associated with idiopathic pulmonary fibrosis through fibroblast proliferation and excessive production of extracellular matrix [63], similar to *TMPRSS2*'s malfunctions in the prostate. TMPRSS4 has been observed contributing to SARS-CoV-2 binding in the small intestine, enabling the virus to enter the intestinal epithelium and spread from there to other tissues through its interaction with ACE2 [52].

Transmembrane protease serine 11A and transmembrane protease serine 11B (TMPRSS11A/B)

TMPRSS11A and 11B belong to the differentially expressed in squamous cell cancer (DESC) subfamily of type II transmembrane serine proteases (TTSPs) [64]. Like the rest of the TTSPs, they are present in a precursor form that needs to be proteolytically cleaved at an activation site to be converted to an active enzyme. One distinct feature of TMPRSS11A is that it undergoes intracellular autocatalysis for zymogen activation before the protein reaches the cell surface, which is different from the extracellular activation demonstrated for other TTSPs [65]. The proteins are expressed on the surface of airway epithelial cells; lymphoid tissue; tongue, esophageal, cervical, and uterine tissue; and are associated with cancers of the lung, head, and neck, to name a few. Interestingly, TMPRSS11A/B have been implicated in playing a role in the entry of several coronaviruses, including SARS [66], MERS [67], and SARS-CoV-2 [26,65,68]. The proteases facilitate virus entry and cell–cell fusion by cleaving the spike protein between the S1/S2 and S2′ sites and activating it. In addition, genetic polymorphisms in the *TMPRSS11A* gene associated with a potential posttranslational modification (rs353163-Arg290Gln and rs13901019-Lys48Arg) could affect viral entry and may be used for association studies in SARS-CoV-2 infection [69].

References

1. Choy K-T, AY-L Wong, P Kaewpreedee, et al. (2020) Remdesivir, lopinavir, emetine, and homoharringtonine inhibit SARS-CoV-2 replication in vitro. *Antiviral Res.* 178:104786.
2. Caly L, JD Druce, MG Catton, et al. (2020) The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. *Antiviral Res.* 178:104787.
3. Guo H, B-J Hu, X-L Yang, et al. (2020) Evolutionary arms race between virus and host drives genetic diversity in bat SARS related coronavirus spike genes. *bioRxiv*:2020.05.13.093658.
4. Valitutto MT, O Aung, KYN Tun, et al. (2020) Detection of novel coronaviruses in bats in Myanmar. *PLOS ONE* 15(4):e0230802.
5. Jackwood MW (2006) The relationship of severe acute respiratory syndrome coronavirus with avian and other coronaviruses. *Avian Dis.* 50(3):315–320.
6. Kim OJ, DH Lee, CH Lee (2006) Close relationship between SARS-coronavirus and group 2 coronavirus. *J. Microbiol.* 44(1):83–91.
7. Wu A, Y Peng, B Huang, et al. (2020) Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. *Cell Host Microbe* 27(3):325–328.
8. Oberemok VV, KV Laikova, KA Yurchenko, et al. (2020) SARS-CoV-2 will continue to circulate in the human population: an opinion from the point of view of the virus-host relationship. *Inflamm. Res.* 69(7):635–640.
9. Donoghue M, F Hsieh, E Baronas, et al. (2000) A novel angiotensin-converting enzyme–related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ. Res.* 87(5):e1–e9.
10. Hamming I, W Timens, MLC Bulthuis, et al. (2004) Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J. Pathol.* 203(2):631–637.
11. Kabbani N, JL Olds (2020) Does COVID19 infect the brain? If so, smokers might be at a higher risk. *Mol. Pharmacol.* 97(5):351.
12. Kuba K, Y Imai, JM Penninger (2013) Multiple functions of angiotensin-converting enzyme 2 and its relevance in cardiovascular diseases. *Circ. J.* 77(2):301–308.
13. Wang H, P Yang, K Liu, et al. (2008) SARS coronavirus entry into host cells through a novel clathrin- and caveolae-independent endocytic pathway. *Cell Res.* 18(2):290–301.
14. Diaz JH (2020) Hypothesis: angiotensin-converting enzyme inhibitors and angiotensin receptor blockers may increase the risk of severe COVID-19. *J. Travel Med.* 27(3):taaa041.
15. Licitra BN, GR Whittaker, EJ Dubovi, et al. (2014) Genotypic characterization of canine coronaviruses associated with fatal canine neonatal enteritis in the United States. *J. Clin. Microbiol.* 52(12):4230–4238.
16. Whitworth KM, RRR Rowland, V Petrovan, et al. (2019) Resistance to coronavirus infection in amino peptidase N-deficient pigs. *Transgenic Res.* 28(1):21–32.
17. Moore BD, RU Jin, L Osaki, et al. (2015) Identification of alanyl aminopeptidase (CD13) as a surface marker for isolation of mature gastric zymogenic chief cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* 309(12):G955–G964.
18. Qi F, S Qian, S Zhang, et al. (2020) Single cell RNA sequencing of 13 human tissues identify cell types and receptors of human coronaviruses. *Biochem. Biophys. Res. Commun.* 526(1):135–140.
19. Grass GD, BP Toole (2015) How, with whom and when: an overview of CD147-mediated regulatory networks influencing matrix metalloproteinase activity. *Biosci Rep* 36(1):e00283.
20. Chen Z et al. (2005) Function of HAb18G/CD147 in invasion of host cells by severe acute respiratory syndrome coronavirus. *J Infect Dis* 191(5):755–60.
21. Wang K et al. (2020) CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. *Signal Transduct Target Ther* 5(1):283.
22. Rahimi N (2020) C-type lectin CD209L/L-SIGN and CD209/DC-SIGN: cell adhesion molecules turned to pathogen recognition receptors. *Biology* (Basel):10(1).
23. Liu W et al. (2004) Characterization of a novel C-type lectin-like gene, LSECtin: demonstration of carbohydrate binding and expression in sinusoidal endothelial cells of liver and lymph node. *J Biol Chem* 279(18):18748–18758.
24. Kizuka Y et al. (2015) Clec4g (LSECtin) interacts with BACE1 and suppresses Abeta generation. *FEBS Lett* 589(13):1418–1422.
25. Gramberg T et al. (2005) LSECtin interacts with filovirus glycoproteins and the spike protein of SARS coronavirus. *Virology* 340(2):224–36.
26. Singh M, Bansal V, Feschotte C (2020) A single-cell RNA expression map of human coronavirus entry factors. *Cell Rep* 32(12):108175.
27. Powlesland AS et al. (2008) A novel mechanism for LSECtin binding to Ebola virus surface glycoprotein through truncated glycans. *J Biol Chem* 283(1):593–602.
28. Domínguez-Soto A et al. (2009) The pathogen receptor liver and lymph node sinusoidal endothelial cell C-type lectin is expressed in human Kupffer cells and regulated by PU.1. *Hepatology* 49(1):287–96.
29. Chen L, S Zheng (2020) Understand variability of COVID-19 through population and tissue variations in expression of SARS-CoV-2 host genes. *Inform Med Unlocked* 21:100443.
30. Houseweart MK, LA Pennacchio, A Vilaythong, et al. (2003) Cathepsin B but not cathepsins L or S contributes to the pathogenesis of Unverricht-Lundborg progressive myoclonus epilepsy (EPM1). *J. Neurobiol.* 56(4):315–327.
31. Moon HY, A Becke, D Berron, et al. (2016) Running-induced systemic cathepsin B secretion is associated with memory function. *Cell Metab.* 24(2):332–340.
32. Fais S (2007) Cannibalism: A way to feed on metastatic tumors. *Cancer Lett.* 258(2):155–164.
33. Hook GR, J Yu, N Sipes, et al. (2013) The cysteine protease cathepsin B Is a key drug target and cysteine protease inhibitors are potential therapeutics for traumatic brain injury. *J. Neurotrauma* 31(5):515–529.
34. Hook VYH, M Kindy, G Hook (2008) Inhibitors of cathepsin B improve memory and reduce β-amyloid in transgenic Alzheimer disease mice expressing the wild-type, but not the Swedish mutant, β-secretase site of the amyloid precursor protein. *J. Biol. Chem.* 283(12):7745–7753.
35. Smieszek SP, BP Przychodzen, MH Polymeropoulos (2020) Amantadine disrupts lysosomal gene expression; potential therapy for COVID19. *bioRxiv*:2020.04.05.026187.
36. Helms J, S Kremer, H Merdji et al. (2020) Neurologic features in severe SARS-CoV-2 infection. *N. Engl. J. Med.* 382(23):2268–2270.
37. Bosch BJ, W Bartelink, PJM Rottier (2008) Cathepsin L functionally cleaves the severe acute respiratory syndrome coronavirus class I fusion protein upstream of rather than adjacent to the fusion peptide. *J. Virol.* 82(17):8887.
38. Raj VS, H Mou, SL Smits, et al. (2013) Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature* 495(7440):251–254.
39. Solerte SB, A Di Sabatino, M Galli, et al. (2020) Dipeptidyl peptidase-4 (DPP4) inhibition in COVID-19. *Acta Diabetol.*:1–5.
40. Zheng SL, AJ Roddick, R Aghar-Jaffar, et al. (2018) Association between use of sodium-glucose cotransporter 2 inhibitors, glucagon-like peptide 1 agonists, and dipeptidyl peptidase 4 inhibitors with all-cause mortality in patients with type 2 diabetes. *JAMA* 319(15):1580–1591.
41. Shiryaev SA, AG Remacle, BI Ratnikov, et al. (2007) Targeting host cell furin proprotein convertases as a therapeutic strategy against bacterial toxins and viral pathogens. *J. Biol. Chem.* 282(29):20847–20853.
42. Hoffmann M, H Kleine-Weber, S Pöhlmann (2020) A multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. *Mol. Cell* 78(4):779–784.e5.
43. Coutard B, C Valle, X de Lamballerie, et al. (2020) The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. Antiviral Res. 176:104742.
44. Fantin A, Maden CH, Ruhrberg C (2009) Neuropilin ligands in vascular and neuronal patterning. *Biochem Soc Trans* 37(Pt 6):1228–1232.
45. Wang HB et al. (2015) Neuropilin 1 is an entry factor that promotes EBV infection of nasopharyngeal epithelial cells. *Nat Commun* 6:6240.
46. Cantuti-Castelvetri L et al. (2020) Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. *Science* 370(6518):856–860.
47. Daly JL et al. (2020) Neuropilin-1 is a host factor for SARS-CoV-2 infection. *Science* 370(6518):861–865.
48. Hedstrom L (2002) Serine protease mechanism and specificity. *Chem. Rev.* 102(12):4501–4524.
49. Lucas JM, C Heinlein, T Kim, et al. (2014) The androgen-regulated protease TMPRSS2 activates a proteolytic cascade involving components of the tumor microenvironment and promotes prostate cancer metastasis. *Cancer Discov.* 4(11):1310–1325.

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50. Wilson S, B Greer, J Hooper, et al. (2005) The membrane-anchored serine protease, TMPRSS2, activates PAR-2 in prostate cancer cells. *Biochem. J.* 388(3):967–972.
51. Hoffmann M, H Kleine-Weber, S Schroeder, et al. (2020) SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181(2):271-280.e8.
52. Zang R, MFG Castro, BT McCune, et al. (2020) TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. *Sci. Immunol.* 5(47):eabc3582.
53. Wallrapp C, S Hähnel, F Müller-Pillasch, et al. (2000) A novel transmembrane serine protease (TMPRSS3) overexpressed in pancreatic cancer. *Cancer Res.* 60(10):2602–2606.
54. Takahashi T, H Uehara, K Izumi (2013) Inhibitory effect of soluble EP2 receptor on ovarian tumor growth in nude mice and utility of TMPRSS4 as a combinatorial molecular target. *Int. J. Oncol.* 43(2):416–424.
55. Ohler A, C Becker-Pauly (2011) Morpholino knockdown of the ubiquitously expressed transmembrane serine protease TMPRSS4a in zebrafish embryos exhibits severe defects in organogenesis and cell adhesion. *Biol. Chem.* 392(7):653–664.
56. Kim S, HY Kang, E-H Nam, et al. (2010) TMPRSS4 induces invasion and epithelial-mesenchymal transition through upregulation of integrin alpha5 and its signaling pathways. *Carcinogenesis* 31(4):597–606.
57. Larzabal L, PA Nguewa, R Pio, et al. (2011) Overexpression of TMPRSS4 in non-small cell lung cancer is associated with poor prognosis in patients with squamous histology. *Br. J. Cancer* 105(10):1608–1614.
58. Liang B, M Wu, Y Bu, et al. (2013) Prognostic value of TMPRSS4 expression in patients with breast cancer. *Med. Oncol.* 30(2):497.
59. Cheng D, B Liang, Y Li (2013) High TMPRSS4 expression is a predictor of poor prognosis in cervical squamous cell carcinoma. *Cancer Epidemiol.* 37(6):993–997.
60. Wu X-Y, L Zhang, K-M Zhang, et al. (2014) Clinical implication of TMPRSS4 expression in human gallbladder cancer. *Tumour Biol.* 35(6):5481–5486.
61. Sheng H, W Shen, J Zeng, et al. (2014) Prognostic significance of TMPRSS4 in gastric cancer. *Neoplasma* 61(2):213–217.
62. Li T, Z-C Zeng, L Wang, et al. (2011) Radiation enhances long-term metastasis potential of residual hepatocellular carcinoma in nude mice through TMPRSS4-induced epithelial-mesenchymal transition. *Cancer Gene Ther.* 18(9):617–626.
63. Valero-Jiménez A, J Zúñiga, J Cisneros, et al. (2018) Transmembrane protease, serine 4 (TMPRSS4) is upregulated in IPF lungs and increases the fibrotic response in bleomycin-induced lung injury. *PLOS ONE* 13(3):e0192963.
64. Bugge TH, TM Antalis, Q Wu (2009) Type II transmembrane serine proteases. *J Biol Chem* 284(35):23177–23181.
65. Zhang C et al. (2020) Intracellular autoactivation of TMPRSS11A, an airway epithelial transmembrane serine protease. *J Biol Chem* 295(36):12686–12696.
66. Kam YW et al. (2009) Cleavage of the SARS coronavirus spike glycoprotein by airway proteases enhances virus entry into human bronchial epithelial cells *in vitro*. *PLoS One* 4(11):e7870.
67. Zmora P et al. (2018) TMPRSS11A activates the influenza A virus hemagglutinin and the MERS coronavirus spike protein and is insensitive against blockade by HAI-1. *J Biol Chem* 293(36):13863–13873.
68. Fuentes-Prior P (2020) Priming of SARS-CoV-2 S protein by several membrane-bound serine proteinases could explain enhanced viral infectivity and systemic COVID-19 infection. *J Biol Chem* 296:100135.
69. Vargas-Alarcón G, R Posadas-Sánchez, J Ramírez-Bello (2020) Variability in genes related to SARS-CoV-2 entry into host cells (*ACE2*, *TMPRSS2*, *TMPRSS11A*, *ELANE*, and *CTSL*) and its potential use in association studies. *Life Sciences* 260:118313.

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