HHS Public Access

Author manuscript

J Mol Endocrinol. Author manuscript; available in PMC 2023 November 02.

Published in final edited form as:

J Mol Endocrinol. ; 69(2): R63–R79. doi:10.1530/JME-21-0282.

Going Viral in the Islet: Mediators of SARS-CoV-2 Entry Beyond ACE2

Rohita Rangu[1,2], **Pandora L. Wander**[1,3], **Breanne M. Barrow**[1], **Sakeneh Zraika**[1,2]

[1]Veterans Affairs Puget Sound Health Care System, Seattle, WA 98108, United States

[2]Division of Metabolism, Endocrinology and Nutrition, Department of Medicine, University of Washington, Seattle, WA 98195, United States

[3]Division of General Internal Medicine, Department of Medicine, University of Washington, Seattle, WA 98195, United States

Abstract

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Following initial infection of airway epithelia, SARS-CoV-2 invades a wide range of cells in multiple organs, including pancreatic islet cells. Diabetes is now recognized as a risk factor for severe COVID-19 outcomes, including hospitalisation and death. Additionally, COVID-19 is associated with higher risk of new-onset diabetes and metabolic complications of diabetes. One mechanism by which these deleterious outcomes may occur is via destruction of insulin-producing islet β cells, either directly by SARS-CoV-2 entry into β cells or indirectly due to inflammation and fibrosis in the surrounding microenvironment. While the canonical pathway of viral entry via angiotensin converting enzyme 2 (ACE2) has been established as a major route of SARS-CoV-2 infection in the lung, it may not be solely responsible for viral entry into the endocrine pancreas. This is likely due to divergent expression of viral entry factors amongst different tissues. For example, expression of ACE2 has not been unequivocally demonstrated in β cells. Thus, it is important to understand how other proteins known to be highly expressed in pancreatic endocrine cells may be involved in SARS-CoV-2 entry, with the view that these could be targeted to prevent demise of the β cell in COVID-19. To that end, this review discusses alternate receptors of SARS-CoV-2 (CD147 and GRP78), as well as mediators (furin, TMPRSS2, cathepsin L, ADAM17, neuropilin-1 and heparan sulphate) that may facilitate SARS-CoV-2 entry into pancreatic islets independent of or in conjunction with ACE2.

Keywords

COVID-19; SARS-CoV-2; islet; ACE2; diabetes; furin; TMPRSS2; cathepsin L; ADAM17; GRP78; NRP1; CD147; heparan sulfate

Correspondence to: Sakeneh Zraika, PhD, 1660 South Columbian Way (151), Seattle, WA 98108, USA, Tel: 206-768-5391 / Fax: 206-764-2164, zraikas@uw.edu.

Author Contribution Statement

R.R. and S.Z. conceived the idea for the manuscript. R.R., P.L.W. and S.Z. wrote the manuscript. P.L.W., B.B. and S.Z. edited the manuscript.

Declaration of Interest

The authors have no conflicts of interest to declare.

Introduction

COVID-19 and Diabetes

In 2019, 9.3% of the world's population was estimated to have diabetes, a number that is predicted to increase by 25% within the next ten years (Saeedi *et al.* 2019). At the same time, despite availability of effective vaccines, SARS-CoV-2 infections are predicted to continue around the world (Telenti *et al.* 2021). These two pandemics are closely linked, and each condition may contribute to the global burden of the other. Prevalent diabetes is an independent risk factor for hospitalisation and death among individuals with COVID-19 (Wander *et al.* 2021). Conversely, a positive test for SARS-CoV-2 is associated with a 40% higher risk of new-onset diabetes and increased use of glucose-lowering medications, including insulin (Al-Aly *et al.* 2021; Wander *et al.* 2022; Xie & Al-Aly 2022). Moreover, an excess burden of metabolic complications commonly associated with diabetes is observed within months of COVID-19 infection. These sequelae include disorders of lipid metabolism and obesity (Al-Aly *et al.* 2021). Such outcomes are exacerbated in hospitalised individuals with COVID-19, where risks are highest in those admitted to intensive care (Al-Aly *et al.* 2021).

While islet cell autoantibodies are rarely detected, new-onset diabetes after COVID-19 appears to be frequently complicated by diabetic ketoacidosis (DKA) (Misra *et al.* 2021). New-onset diabetes that presents with DKA has been recognized as ketosis-prone and is marked by significant impairment of insulin secretion and insulin action at presentation (Vellanki & Umpierrez 2017). Together, these lines of evidence suggest that early β-cell injury may contribute to the pathogenesis of new-onset diabetes after COVID-19; however, mechanisms contributing to such β-cell injury, including the critical pathways mediating viral entry, remain poorly characterised.

SARS-CoV-2 Enters Human Pancreatic Endocrine Cells

Studies investigating viral entry into islet α and β cells have done so using either SARS-CoV-2 itself, or a pseudovirus where the envelope glycoprotein is replaced by the SARS-CoV-2 spike protein. In human islets, SARS-CoV-2 was shown to enter α and β cells *ex vivo* (Yang *et al.* 2020; Müller *et al.* 2021), with viral infiltrates detected mostly in insulin-positive β cells (Wu *et al.* 2021). Although considered functionally immature, human pluripotent stem cell-derived α and β cells were permissive to SARS-CoV-2 pseudovirus infection *in vitro* and *in vivo* when xenografted into mice (Yang *et al.* 2020). Further, immunohistochemical and molecular analyses showed SARS-CoV-2 infiltration in pancreas from humans with COVID-19, particularly in β cells, sometimes more so than in other pancreatic endocrine cells (Müller *et al.* 2021; Steenblock *et al.* 2021; Tang *et al.* 2021a; Wu *et al.* 2021). In sum, there is convincing evidence that SARS-CoV-2 directly infects the islet.

Following infection, SARS-CoV-2 causes morphological, transcriptional, and functional derangements in islet cells (Mine et al. 2022). For example, dilatation and vacuolization of the endoplasmic reticulum (ER)–Golgi apparatus complex was evident in SARS-CoV-2-infected islet cells (Müller *et al.* 2021). At the transcriptional level, loss of β-cell identity

was observed, wherein α- and acinar cell markers were increased and insulin was decreased in SARS-CoV-2- vs. mock-infected β cells (Müller *et al.* 2021; Tang *et al.* 2021a). Insulin protein was also decreased in SARS-CoV-2-infected β cells (Tang *et al.* 2021a), as was number of insulin secretory granules, C-peptide and insulin immunoreactivity, and glucose-stimulated insulin secretion (Müller *et al.* 2021). It is suggested these perturbations may be mediated by ER stress (Müller *et al.* 2021). SARS-CoV-2 infection of islets also upregulated several interferon-stimulated genes (Müller *et al.* 2021), indicative of an inflammatory response that may exacerbate cellular damage.

ACE2-mediated SARS-CoV-2 Entry

Angiotensin converting enzyme 2 (ACE2) is the canonical receptor of SARS-CoV-2. Following binding of SARS-CoV-2 to ACE2, there are two routes for viral entry into host cells: direct membrane fusion and endocytosis.

Host cell membrane fusion pathway

SARS-CoV-2 contains a transmembrane spike glycoprotein comprising the S1 and S2 subunits. S1 binds to the host cell receptor and S2 mediates fusion of the viral and host cell membranes. As illustrated in Fig. 1, SARS-CoV-2 entry begins with binding of the spike protein to ACE2 (Hoffmann *et al.* 2020a). SARS-CoV-2-spike must be sequentially cleaved along the S1/S2 junction, usually by furin (Hoffmann *et al.* 2020b), and at a second site (S2') by host serine proteases, typically transmembrane serine protease 2 (TMPRSS2) (Hoffmann *et al.* 2020a) (Fig. 1). This exposes hydrophobic amino acid residues in processed S2 that embed themselves into the host cell membrane, further facilitating fusion of the viral envelope and host cell membrane (Huang *et al.* 2020) (Fig. 1).

Alternative endocytosis pathway

Alternatively, SARS-CoV-2-spike binding to ACE2 is followed by uptake of virions into endosomes. Cathepsin L (CTSL), a pH-sensitive endosomal protease, primes the spike by cleaving it into smaller fragments after the initial furin-mediated S1/S2 cleavage (Zhao *et al.* 2021). The viral envelope then fuses with the endosomal membrane, releasing viral machinery and genetic material.

Although the membrane fusion pathway is 100–1000 times more efficient than the endocytosis pathway (as measured for SARS-CoV) (Matsuyama *et al.* 2005), the mode of viral entry amongst different cells is dependent on protease expression (Padmanabhan *et al.* 2020).

ACE2 Expression in α and β Cells

While the literature is largely in agreement that SARS-CoV-2 enters the islet, the mechanism of viral entry is still being debated. A major point of uncertainty is whether ACE2 is expressed in α and β cells (El-Huneidi *et al.* 2021). Data in this regard have derived mostly from RNA-sequencing (RNA-seq) and immunohistochemistry analyses.

RNA-sequencing—Two studies demonstrated ACE2 expression in α and β cells, one via single-cell RNA-seq (scRNA-seq) on adult human islets (Yang *et al.* 2020), and the other by analysing existing scRNA-seq datasets (Lazartigues *et al.* 2020). However, most of the data from a total of 11 bulk and single-cell RNA-seq datasets show little ACE2 expression in α or β cells (Coate *et al.* 2020; Kusmartseva *et al.* 2020; Lee *et al.* 2020; Liu *et al.* 2020; Qadir *et al.* 2021; Wu *et al.* 2021) – and quantitatively much lower expression than key genes enriched in α (e.g. *GCG, ARX, IRX2*) and β (e.g. *INS, PDX1, MAFA*) cells (Coate *et al.* 2020).

Immunohistochemistry—Among studies showing positive ACE2 staining in islets, there is general agreement that β cells express more ACE2 than α cells. In fact, β cells had the highest frequency of ACE2 staining among pancreatic endocrine cell types (Müller *et al.* 2021). One paper described islet ACE2 immunofluorescence as weak and diffuse, arising from a subset of cells that were identified to be β cells and not α cells (Fignani *et al.* 2020). Further, ACE2 in β cells was predominantly found in insulin secretory granules, with some expression on the plasma membrane (Fignani *et al.* 2020). Interestingly, a study that utilized various antibodies raised against different regions of ACE2 found that the prevalent ACE2 isoform in β cells is short-ACE2 (Fignani *et al.* 2020). Given that short-ACE2 lacks the amino acid residues required to bind SARS-CoV-2-spike (Onabajo *et al.* 2020), it is unlikely to be responsible for viral entry. Finally, two larger cohort studies utilising multiple anti-ACE2 antibodies on pancreatic sections from female and male donors with and without SARS-CoV-2 infection and a range of ages and BMIs found that pancreatic endocrine cells exhibited little/no ACE2 expression (Coate *et al.* 2020; Kusmartseva *et al.* 2020). Despite extensive validation, the commonly used anti-ACE2 antibodies produce vastly different staining patterns amongst various studies (Yang *et al.* 2010; Brar *et al.* 2017; Coate *et al.* 2020; Fignani *et al.* 2020; Hikmet *et al.* 2020; Kusmartseva *et al.* 2020; Lazartigues *et al.* 2020; Yang *et al.* 2020; Müller *et al.* 2021; Qadir *et al.* 2021; Steenblock *et al.* 2021; Wu *et al.* 2021). These differences could not be explained by β-cell maturity level or technical aspects, like antibody dilution (Coate *et al.* 2020).

What may be contributing to the variability in islet ACE2 expression across studies of human donors? One factor may be differences in clinical characteristics such as age, sex, and presence of SARS-CoV-2 infection. Another important factor, particularly in SARS-CoV-2 positive donors, is presence of pro-inflammatory cytokines, which can upregulate β-cell/islet ACE2 expression (Fignani *et al.* 2020). Since cytokine levels change over the course of COVID-19 disease, the timing of sample collection in relation to onset of illness in SARS-CoV-2 positive donors is a critical consideration for assessing ACE2 expression.

The cytokine milieu to which islets are exposed may influence the ability of ACE2 to mediate SARS-CoV-2 entry. In Fig. 2, potential scenarios are presented for SARS-CoV-2 entry into islet cells in the setting of high vs. low ACE2 expression, taking into account the roles of other viral entry mediators. When ACE2 is sufficiently expressed, cofactors and co-receptors may assist ACE2-mediated entry, and alternative receptors may work in parallel to increase the rate of viral entry. If islet ACE2 levels remain too low to permit viral entry, alternative receptors may be primarily responsible for SARS-CoV-2 entry into islet cells. Additionally, viral invasion of the islet may induce a local inflammatory response

(Müller *et al.* 2021). This may affect expression of entry mediators (Chu *et al.* 2018; Cantuti-Castelvetri *et al.* 2020), potentially altering the mode of subsequent infection by virions into the same or surrounding host cells so that it differs from that of the original infection.

The following sections outline the roles of different viral entry mediators, which may function as, or alongside, SARS-CoV-2 receptors (Fig. 3). Thus far, many studies of SARS-CoV-2 entry have utilized lung tissue or related models; this review aims to contextualise data on entry mediators to the endocrine pancreas.

Alternative SARS-CoV-2 Receptors

Cluster of differentiation 147

Function—Cluster of differentiation 147 (CD147), also known as basigin or extracellular matrix metalloprotease inducer, is a widely expressed member of the immunoglobulin superfamily. It is predominantly membrane bound and highly glycosylated. It is thought to be involved in cell-cell recognition, although its role in inducing extracellular matrix metalloproteases is most extensively studied. CD147 was found to be increased in pulmonary fibrosis (Guillot *et al.* 2006), and inhibition of CD147 reduced differentiation of fibroblasts to myofibroblasts (Ulrich & Pillat 2020). This raises questions about its possible role in islet fibrosis, especially with COVID-19.

Expression in the islet—CD147 is very highly expressed in α, β, and γ cells (Uhlén *et al.* 2015; Segerstolpe *et al.* 2016), though one study reported it absent in islets (Zhao *et al.* 2001). Though not reported in islets, CD147 is upregulated in various cell types upon ER (Grass & Toole 2016) and oxidative (Ke *et al.* 2012) stress, which occur in patients with COVID-19 (Rosa-Fernandes *et al.* 2021).

Role in SARS-CoV-2 infection—The ability of CD147 to serve as an alternative SARS-CoV-2 receptor is exemplified by the demonstration that cells otherwise not susceptible to viral infection allowed SARS-CoV-2 (and pseudovirus) entry upon CD147 expression (Wang *et al.* 2020). Additionally, humanised CD147 mice infected with SARS-CoV-2 had detectable viral loads in their lungs, unlike virus-infected wild-type mice (Wang *et al.* 2020; Geng *et al.* 2021). Some (Wang *et al.* 2020; Geng *et al.* 2021), but not all (Ragotte *et al.* 2021; Shilts *et al.* 2021), studies provide evidence that SARS-CoV-2-spike binds to CD147. CD147-SARS-CoV-2-spike binding is also supported by molecular modelling data (Helal *et al.* 2020), and evidence of CD147 and SARS-CoV-2-spike colocalization in lung and kidney tissue from donors with COVID-19 (Wang *et al.* 2020). Further, pseudovirus infection of CD147-expressing cells could be neutralized by addition of the extracellular domain of CD147, suggesting it competes with membrane-bound CD147 for spike binding (Wang *et al.* 2020). In intervention studies, CD147 overexpression increased SARS-CoV-2 infection (Wang *et al.* 2020), whereas CD147 blockade/knockdown had the opposite effect in most (Wang *et al.* 2020; Fenizia *et al.* 2021; Geng *et al.* 2021), but not all (Ragotte *et al.* 2021) studies.

CD147 facilitates SARS-CoV-2 entry into host cells via endocytosis (Wang *et al.* 2020). It does not bind ACE2 (Wang *et al.* 2020); however, CD147 silencing reduced ACE2 protein levels (Fenizia *et al.* 2021), suggesting an interaction between the two proteins that is yet to be understood. After SARS-CoV-2 infection, CD147 was upregulated in human airway epithelial cells (Fenizia *et al.* 2021)— a response that may exacerbate virus entry.

Concluding remarks—CD147 may serve as an alternative SARS-CoV-2 receptor (Fig. 3A)—as opposed to a cofactor/co-receptor, especially as it promotes SARS-CoV-2 infection under conditions in which (human) ACE2 receptor is not expressed. It may also act via other mechanisms to permit viral infection. CD147 is worthy of study in the mediation of SARS-CoV-2 entry in islet cells with and without high ACE2 expression.

Glucose Regulatory Protein 78

Function—Glucose regulatory protein 78 (GRP78) is an ER-localised chaperone that regulates ER signalling molecules to ensure proper protein folding. During ER stress, the unfolded protein response upregulates GRP78. However, GRP78 recycling from the Golgi system to the ER becomes saturated and GRP78 is missorted to the cell surface. Thus, cell surface GRP78 (csGRP78) can act as a receptor and may be involved in signal transduction (Ibrahim *et al.* 2019). Although ER and plasma membrane GRP78 are most commonly studied, GRP78 also exists as mitochondrial, cytosolic, secreted, and nuclear proteins (Ni *et al.* 2011).

Expression in the islet—GRP78 is expressed in all pancreatic cell types, including β cells (Uhlén *et al.* 2015; Segerstolpe *et al.* 2016). Some (Allagnat *et al.* 2012; Brozzi *et al.* 2015), but not all (Cardozo *et al.* 2005; Pirot *et al.* 2006; Åkerfeldt *et al.* 2008) studies show GRP78 to be upregulated in β cells under cytokine stress. Further, GRP78 is increased in diabetic mouse models (Laybutt *et al.* 2007; Wang *et al.* 2009) and islets from donors with type 2 diabetes (T2D) compared to non-diabetic donors (Laybutt *et al.* 2007; Hull *et al.* 2009). However, some studies find no significant increase in islet GRP78 levels in T2D donors (Marchetti *et al.* 2007; Marselli *et al.* 2020).

Role in SARS-CoV-2 infection—GRP78 may promote viral infection by serving as an alternative receptor and/or stabilising spike-receptor binding ((Ha *et al.* 2020); Fig. 3A). *In silico* modelling predicted that GRP78 docks to the receptor-binding domain of SARS-CoV-2-spike (Ibrahim *et al.* 2020), which has the same GRP78 recognition site as other human and bat coronaviruses (Elfiky 2020). GRP78 binds MERS-CoV-spike *in vitro*, and blockade/knockdown of GRP78 reduced MERS-CoV infection (Chu *et al.* 2018). Additionally, GRP78 overexpression increased, but was not sufficient for, MERS-CoV entry into cells (Chu *et al.* 2018). Similar to studies of MERS-CoV, GRP78 can bind SARS-CoV-2 and ACE2 *in vitro* at the ER and cell surface of kidney epithelial cells (Carlos *et al.* 2021). Specifically depleting or blocking csGRP78 decreased SARS-CoV-2 and pseudovirus entry (Carlos *et al.* 2021). Hence, csGRP78 may bind SARS-CoV-2-spike and act as an alternative receptor, cofactor or co-receptor, working in place of or in conjunction with ACE2 to facilitate viral entry.

SARS-CoV-2 infection induces ER stress in host cells (Köseler *et al.* 2020; Rosa-Fernandes *et al.* 2021), likely due to an inability to accommodate the drastic increase in protein production that occurs during viral replication (Aoe 2020). Hence, coronavirus infection typically upregulates GRP78 (Chan *et al.* 2006; Chu *et al.* 2018). In line with this, serum GRP78 levels are increased in COVID-19 patients compared to both COVID-19-negative pneumonia patients and healthy volunteers (Sabirli *et al.* 2021). Under conditions of ER stress, other SARS-CoV-2 entry factors can also modulate GRP78 levels. For example, CD147 mediates ER stress-induced GRP78 upregulation (Tang *et al.* 2012). Thus, CD147 upregulation following ER stress may exacerbate GRP78 mediation of SARS-CoV-2 entry in a feed-forward fashion. Additionally, GRP78 can impact cellular ACE2 levels. GRP78 knockdown decreased cell surface ACE2 (csACE2) (but not total ACE2) independent of ER stress, suggesting that GRP78 may be involved in ACE2 trafficking (Carlos *et al.* 2021). Thus, via several interactions with other entry mediators, GRP78 can influence SARS-COV-2 entry.

Concluding remarks—GRP78 can promote SARS-CoV-2 entry via multiple mechanisms, which may be due in part to its relationship with other viral entry factors. Apart from ER and cell surface GRP78, other GRP78 forms may also support viral infection, necessitating study of their interactions with SARS-CoV-2 and viral entry mediators. Given its high expression in islets, GRP78 is a prime candidate for initiating and/or mediating viral invasion of pancreatic endocrine cells.

Cofactors and Co-receptors in SARS-CoV-2 Entry

Several proteins outlined below work alongside ACE2 to mediate SARS-CoV-2 entry in various cell types. Cofactors furin, TMPRSS2, and CTSL are proteases that prime SARS-CoV-2-spike for entry; ADAM17 exerts proteolytic activity on ACE2; co-receptors neuropilin-1 (NRP1) and heparan sulfate (HS) assist SARS-CoV-2-spike-ACE2 binding. According to existing literature, the primary role of these mediators is to increase the efficiency of ACE2-mediated viral entry. This may make ACE2 a significant contributor to SARS-CoV-2 infection in β cells. Similarly, and what represents a gap in current knowledge, is that these mediators may also work alongside alternative receptors to permit viral entry into islet cells.

Furin

Function—Furin is a transmembrane endoprotease that cleaves a diverse range of proproteins prior to secretion, as part of their maturation process (Thomas 2002). It is localised to the trans-Golgi network, but also cycles between the cell surface and endosomes (Shapiro *et al.* 1997; Thomas 2002). In islet cells, furin controls proliferation and differentiation (Kayo *et al.* 1996), as well as secretory granule acidification (Louagie *et al.* 2008), the latter being critical for β-cell granule maturation and proinsulin-to-insulin conversion.

Expression in the islet—Furin has moderate to high expression in human pancreatic endocrine cells (Uhlén *et al.* 2015; Brouwers *et al.* 2021), including β cells (Sawada

et al. 2000; Segerstolpe *et al.* 2016; Tang *et al.* 2021a), as shown by RNA-seq and immunohistochemistry.

Role in SARS-CoV-2 infection—As a proprotein convertase, furin cleaves and activates many viral proteins (including SARS-CoV-2 proteins (Murgolo *et al.* 2021)), thereby facilitating viral entry into host cells (Fig. 1; Fig. 3B). This cleavage may occur during viral production in an infected cell, or on virus entry into a host cell (Hoffmann *et al.* 2020b; Shang *et al.* 2020; Papa *et al.* 2021). SARS-CoV-2-spike contains a polybasic furin cleavage site (FCS) at the S1/S2 junction (Coutard *et al.* 2020), which is absent in other SARS coronaviruses (Coutard *et al.* 2020). Studies in other viruses show that insertion of a similar polybasic FCS increases virulence (Claas *et al.* 1998). Interestingly, the structure of the FCS suggests that SARS-CoV-2 uses molecular mimicry to hijack host cell machinery for viral entry (Anand *et al.* 2020). *In vitro* studies showed that furin knockout decreased viral production 100-fold (Papa *et al.* 2021). Mutants of SARS-CoV-2-spike resistant to S1/S2 cleavage had significantly reduced entry into TMPRSS2-positive but not TMPRSS2-negative cells, suggesting that furin-mediated cleavage may only be relevant in cells where the membrane fusion pathway predominates over the endocytosis pathway (Hoffmann *et al.* 2020b). Cells with diminished furin activity still exhibited SARS-CoV-2 spike cleavage, albeit at much reduced levels (Papa *et al.* 2021). This is likely attributed to less efficient spike processing by other cellular proteases, such as trypsin, matriptase, and proprotein convertase 1 (Jaimes *et al.* 2020)—of which the last is highly expressed in β cells and thus may be relevant for viral entry in islets.

Concluding remarks—The unique furin cleavage site in SARS-CoV-2 makes viral envelope-host cell membrane fusion more efficient. Since furin is highly expressed in islets, studies are needed to confirm furin-mediated S1/S2 cleavage in islet cells. Moreover, other islet proteases/proprotein convertases may work alongside furin to cleave the SARS-CoV-2 spike protein at the S1/S2 junction (Jaimes *et al.* 2020; Tang *et al.* 2021b), potentially increasing the rate of viral entry.

Transmembrane Serine Protease 2

Function—TMPRSS2 is a membrane-anchored serine protease first characterized as being androgen-regulated in the prostate. It plays an important role in prostate cancer development and progression (Chen *et al.* 2010), but has also been associated with various processes including digestion, tissue remodeling, blood coagulation, fertility and inflammation.

Expression in the islet—TMPRSS2 expression in islets remains somewhat unclear. Moderate-to-high TMPRSS2 expression in human islets was found by immunostaining (Steenblock *et al.* 2021), and corroborated by RNA-seq and microarray analysis (Taneera *et al.* 2020); however, specific cell type was not reported in these studies. Another study showed TMPRSS2 to be highly expressed in a subset of pancreatic endocrine cells enriched in α, β and δ cells (Uhlén *et al.* 2015), while yet another found TMPRSS2 expression in α but not β cells (Segerstolpe *et al.* 2016). In contrast, scRNA-seq of primary human islets found low TMPRSS2 levels in α and β cells (Yang *et al.* 2020). Integrated analysis of six RNA-seq databases found that less than 1.5% of α and β cells expressed TMPRSS2 (Coate

et al. 2020). However, another analysis of five scRNA-seq datasets found that 17% of α and 5.7% of β cells expressed TMPRSS2 (Kusmartseva *et al.* 2020). Overall, TMPRSS2 expression seems to be moderately low in α and β cells, but likely high enough in other islet cell types to contribute to moderate/high expression in the islet as a whole.

Role in SARS-CoV-2 infection—Once SARS-CoV-2-spike is bound to ACE2, TMPRSS2 cleaves the spike protein at the S2' site to facilitate SARS-CoV-2 entry into cells via the membrane fusion pathway (Hoffmann *et al.* 2020a) (Fig. 1). TMPRSS2 can also cleave ACE2 at its cytoplasmic tail, increasing viral uptake through the CTSL/endocytosis pathway (Heurich *et al.* 2014). TMPRSS2 inhibitors were shown to hamper SARS-CoV-2 viral entry. Camostat mesylate, which inhibits serine proteases like TMPRSS2, reduced SARS-CoV-2-spike entry into TMPRSS2-positive cells, but not TMPRSS2-negative cells (Hoffmann *et al.* 2020a). Interestingly, camostat mesylate combined with E-64d (CTSL inhibitor) prohibited viral entry into TMPRSS2-positive cells, whereas E-64d alone did not (Hoffmann *et al.* 2020a). Overall, this suggests that TMPRSS2 expression can compensate for low CTSL activity in SARS-CoV-2-spike processing (Hoffmann *et al.* 2020a). In cells with low/no TMPRSS2 expression, other TMPRSS2-related proteases may cleave SARS-CoV-2-spike (Hoffmann *et al.* 2021). One such protease is TMPRSS4, whose gene expression is highly correlated with ACE2 in lung cells, even more so than TMPRSS2 (Wruck & Adjaye 2020). TMPRSS4 has been shown to promote SARS-CoV-2 entry in intestinal enterocytes by cleaving SARS-CoV-2-spike (Zang *et al.* 2020). Within the pancreas, TMPRSS4 expression seems to be limited to exocrine and endothelial cell types (Segerstolpe *et al.* 2016; Coate *et al.* 2020; Kusmartseva *et al.* 2020; Lee *et al.* 2020), though it is upregulated in pancreatic cancer cells (Katopodis *et al.* 2021). It is therefore possible that TMPRSS4 is involved in mediating SARS-CoV-2 infection in both the exocrine and endocrine pancreas, the latter via islet endothelial cell interactions.

Concluding remarks—The proteolytic activity of TMPRSS2 is important for SARS-CoV-2 viral entry in many cell types, though it is unknown whether this holds true if the entry receptor is not ACE2. Although TMPRSS2 expression in α and β cells may be moderate/low, compensation by other cellular proteases like CTSL may explain permissiveness of α and β cells to SARS-CoV-2 infection.

Cathepsin L

Function—CTSL is a lysosomal cysteine endoprotease, though it is also found in the nucleus and can be secreted. It plays a major role in degrading extracellular, cytoplasmic and nuclear proteins, and is involved in a diverse range of processes such as autophagy, apoptosis, cell cycle regulation, bone resorption, antigen processing, and tumor invasion/metastasis (Yadati *et al.* 2020). It is required for the development of type 1 diabetes (T1D) in mouse models (Maehr *et al.* 2005), and regulates human and mouse islet cell proliferation (Lo *et al.* 2019).

Expression in the islet—CTSL is ubiquitously expressed (Dana & Pathak 2020), consistent with its wide range of cellular functions. It is present in all pancreatic cell types (Segerstolpe *et al.* 2016; Tang *et al.* 2021a) and is moderately expressed in pancreatic

endocrine cells (Tang *et al.* 2021a), including α and β cells (Kusmartseva *et al.* 2020). β-cell CTSL expression in T2D donors was shown to be higher than in non-diabetic donors (Kusmartseva *et al.* 2020).

Role in SARS-CoV-2 infection—In a human hepatoma cell line (Huh7), CTSL levels were elevated following SARS-CoV-2 pseudovirus entry (Zhao *et al.* 2021), while CTSL overexpression per se increased pseudovirus entry (Zhao *et al.* 2021). Conversely, CTSL knockdown in Huh7 cells or pharmacological inhibition in humanised ACE2 mice using E64d decreased SARS-CoV-2 pseudovirus entry (Zhao *et al.* 2021). Similarly, inactivation of CTSL with ammonium chloride reduced SARS-CoV-2 entry, but inhibition was weaker in TMPRSS2-positive versus TMPRSS2-negative cells (Hoffmann *et al.* 2020a). This indicates that TMPRSS2 and CTSL can each compensate for inactivity of the other.

CTSL cleaves SARS-CoV-2-spike downstream of the S1/S2 site, at a region distinct from the TMPRSS2 cleavage site (Murgolo *et al.* 2021) (Fig. 3C). This action of CTSL enhances virus entry (Zhao *et al.* 2021). S1/S2 cleavage site mutations (ΔCS) only reduced SARS-CoV-2 entry in cells utilising the ACE2-TMPRSS2 pathway (Hoffmann *et al.* 2020b). SARS-COV-2-ΔCS replicates faster than wild-type SARS-CoV-2 in cells lacking TMPRSS2 (Peacock *et al.* 2021; Zhu *et al.* 2021), reinforcing a role for CTSL in increased virulence under permissive conditions.

CTSL can be secreted, often under systemic or local inflammatory conditions (Gomes *et al.* 2020). In keeping with this, COVID-19 patients had elevated circulating CTSL levels compared to healthy volunteers, positively correlating with disease severity (Zhao *et al.* 2021). This suggests that circulating CTSL may exacerbate viral entry during inflammation that accompanies COVID-19.

Concluding remarks—SARS-CoV-2 infection and CTSL seem to have a bidirectional relationship, whereby viral infection increases CTSL levels and in turn, CTSL mediates SARS-CoV-2 entry. While largely studied in the context of ACE2, this protease may work similarly with alternative receptors, and is a good candidate for mediating viral entry into pancreatic endocrine cells with little/no TMPRSS2 expression.

A Disintegrin and Metalloprotease 17

Function—A disintegrin and metalloprotease 17 (ADAM17), also known as tumour necrosis factor α converting enzyme, is a membrane anchored protein responsible for proteolysis of several cell surface proteins (including ACE2; Fig. 3A) to enable ‘shedding’ of their ectodomains.

Expression in the islet—ADAM17 is expressed in the islet, including β cells. A subset of pancreatic endocrine cells enriched in α, β, and δ cells shows moderate ADAM17 expression (Uhlén *et al.* 2015). Similarly, in α- and β-cell populations from non-diabetic and/or T2D donors, ADAM17 is expressed moderately (Segerstolpe *et al.* 2016; Coate *et al.* 2020). Furthermore, circulating ADAM17 levels were elevated in patients with COVID-19 (Palacios *et al.* 2021).

Role in SARS-CoV-2 infection—In the case of SARS-CoV, internalisation of the virus-ACE2 complex upregulates ADAM17 expression/activity (Haga *et al.* 2008), as does ER stress (Rzymski *et al.* 2012). GRP78, which is also upregulated under cytokine/ER stress, protects ADAM17 from inhibition by protein disulphide isomerases (Schäfer *et al.* 2017). The increase in ADAM17 activity enhances ACE2 'shedding', which decreases csACE2 (Kuba *et al.* 2005). This may explain the higher soluble ACE2 (sACE2) levels in COVID-19 patients compared to healthy controls (van Lier *et al.* 2021). Thus, shedding may be a cellular defence mechanism, whereby spike-csACE2 binding is reduced (Zipeto *et al.* 2020). However, SARS-CoV-2-sACE2 can enter cells via endocytosis mediated by angiotensin II type 1 receptor (AT1R) or vasopressin receptor 1B (AVPR1B) (Yeung *et al.* 2021), though this has yet to be studied in pancreatic endocrine cells.

While not yet shown with SARS-CoV-2, ADAM17 inhibition significantly attenuated SARS-CoV entry *in vitro*, and reduced viral titres in mice *in vivo* (Haga *et al.* 2010). This appears to conflict with the idea above, where ADAM17 upregulation would reduce viral entry into cells. One explanation is that ADAM17 upregulation may increase the cleavage and maturation of several cytokines (Zipeto *et al.* 2020; Schreiber *et al.* 2021), whose increased levels may upregulate mediators of SARS-CoV-2 entry.

Concluding remarks—While ADAM17 may be capable of either decreasing or increasing SARS-CoV-2 entry into cells, it is unclear which effect predominates. Schreiber et. al reviews ADAM17's possible roles in COVID-19 infection, including entry and post-infection inflammation-mediated damage (Schreiber *et al.* 2021). In sum, more studies are needed to better understand how ADAM17 affects SARS-CoV-2 infection of the islet.

Neuropilin-1

Function—NRP1 is a cell surface receptor involved in angiogenesis and organ development. It exists in two isoforms—one is truncated and secreted (sNRP1), and the other is a transmembrane protein (Gagnon *et al.* 2000). NRP1 has a large extracellular domain that binds ligands in a host of signalling pathways associated with cell migration, growth, and development (Pellet-Many *et al.* 2008). While its cytoplasmic domain has no known signalling sequence, literature on NRP1's capability to signal independently is divided (Pellet-Many *et al.* 2008). Interestingly, minor alleles of NRP1 were associated with T1D in children (Hasan *et al.* 2010).

Expression in the islet—NRP1 is highly expressed in islets (Tang *et al.* 2021a; Wu *et al.* 2021). Within the pancreas, its expression is mostly confined to islets, especially β cells (Hasan *et al.* 2010), but rarely α cells (Hasan *et al.* 2010; Wu *et al.* 2021). NRP1 was found to be upregulated in β cells from humans with COVID-19 (Wu *et al.* 2021).

Role in SARS-CoV-2 infection—Furin-mediated SARS-CoV-2-spike cleavage creates a C-terminal motif (known as CendR) on S1 to which NRP1 binds (Cantuti-Castelvetri *et al.* 2020; Daly *et al.* 2020; Li & Buck 2021). NRP1 stabilises the C-terminus of S1, facilitating more efficient S1/S2 cleavage (Fig. 3B), and allowing S2 to mediate membrane fusion more rapidly (Li & Buck 2021). Specifically inhibiting binding between NRP1 and

SARS-CoV-2-S1-CendR reduces viral infection in various cell types, including human islets (Cantuti-Castelvetri *et al.* 2020; Daly *et al.* 2020; Wu *et al.* 2021)

When infected with SARS-CoV-2, NRP1-negative ACE2-positive HeLa cells exhibited less viral entry than cells expressing both NRP1 and ACE2, but cells lacking ACE2 exhibited no viral entry (Daly *et al.* 2020). In human islets infected with SARS-CoV-2 *ex vivo*, scRNA-seq showed that ACE2- and NRP1-positive cells had more SARS-CoV-2-nucleocapsid transcripts than cells expressing either ACE2 or NRP1 (Tang *et al.* 2021a). ACE2-positive NRP1-negative cells had fewer transcripts than ACE2-negative NRP1-positive cells, and double-negative cells showed little/no infection (Tang *et al.* 2021a). Further, SARS-CoV-2-spike pseudovirus entry was independently facilitated by ACE2 but not TMPRSS2 or NRP1 (Cantuti-Castelvetri *et al.* 2020). Together, these data support a role for NRP1 as a co-receptor that potentiates ACE2-mediated SARS-CoV-2 infection (Kyrou *et al.* 2021), while data on its ability to independently permit viral entry is limited.

Concluding remarks—NRP1 is highly expressed in β cells, where it is upregulated in patients with COVID-19 and may serve to exacerbate infection. Its inhibition in human islets reduced SARS-CoV-2 infection, making it a good therapeutic drug target. While NRP1 facilitates SARS-CoV-2 entry as a co-receptor with ACE2 in various cell types, it may function similarly with alternative receptors that are present in islets – this is an area of investigation that requires further study.

Heparan sulfate

Function—HS exists as a highly acidic, negatively charged, unbranched polysaccharide and is either conjugated to proteins (HS proteoglycans; HSPGs) or remains unconjugated. Present in all cell types, both HS and HSPGs act as cell surface co-receptors, altering ligand binding affinity. They also maintain extracellular matrix structure, thereby playing a role in cell-cell adhesion and angiogenesis. In mouse islets, HS was shown to be important for β-cell maturation and insulin secretion (Takahashi *et al.* 2009), as well as β-cell survival and protection from autoimmunity and T1D (Ziolkowski *et al.* 2012).

Expression in the islet—HS exhibits high expression in β cells and islets (Ziolkowski *et al.* 2012). Mouse models of T2D had lower islet HS and HSPG levels than wild-type mice (Dhounchak *et al.* 2021).

Role in SARS-CoV-2 infection—HS binds SARS-CoV-2-spike (Clausen *et al.* 2020) through its receptor-binding domain (Clausen *et al.* 2020). This increases proximity of SARS-CoV-2 to the host cell membrane. The heparan-binding site on SARS-CoV-2-spike is adjacent to, but distinct from, the ACE2-binding residues (Clausen *et al.* 2020). Hence, SARS-CoV-2-spike may bind both ACE2 and HS simultaneously (Clausen *et al.* 2020) (Fig. 3B). After binding to HS, the receptor-binding domain of SARS-CoV-2-spike undergoes a conformational change (Mycroft-West *et al.* 2020), which may increase the number of spike proteins bound to any one ACE2 receptor (Clausen *et al.* 2020).

Treatment with heparinases (Clausen *et al.* 2020) and disruption of genes involved in HSPG biosynthesis (Clausen *et al.* 2020; Zhang *et al.* 2020) markedly reduced SARS-

CoV-2 viral infection rates, without impacting ACE2 levels (Clausen *et al.* 2020) or cell viability (Clausen *et al.* 2020; Zhang *et al.* 2020). HS-assisted viral entry requires the actin cytoskeleton, consistent with HS-assisted endocytosis (Zhang *et al.* 2020).

Concluding remarks—HS is highly expressed in β cells/islets, where it could mediate SARS-CoV-2 infection as a co-receptor. There is evidence that SARS-CoV-2 entry is enhanced by formation of the ACE2-SARS-CoV-2-HS complex. Since HS does not directly bind ACE2, it is a good candidate for studies investigating SARS-CoV-2 recruitment to the cell surface when alternative receptors are involved.

Other mediators of interest

Several other proteins may mediate SARS-CoV-2 entry in cells that do not utilise the ACE2/TMPRSS2 pathway. One such protein is transferrin receptor (TFRC), a cell surface receptor responsible for endocytosis of iron. TFRC is highly expressed in islets, and is present in β cells (Segerstolpe *et al.* 2016; Wu *et al.* 2021). TFRC directly binds SARS-CoV-2-spike (Tang *et al.* 2020), and blocking this binding significantly reduces viral entry (Tang *et al.* 2020). Since TFRC shuttles between the cytoplasm and plasma membrane, it may transport SARS-CoV-2 in a similar fashion (Tang *et al.* 2020).

Dipeptidyl peptidase-4 (DPP-4), along with being the MERS-CoV receptor, is a target for several T2D medications. While DPP-4 is expressed in the islet, there is evidence to both support (Bugliani *et al.* 2018; Steenblock *et al.* 2021) and refute (Omar *et al.* 2014; Segerstolpe *et al.* 2016) its expression in β cells. Some (Li *et al.* 2020; Vankadari & Wilce 2020), but not all (Cameron *et al.* 2021) *in silico* studies find DPP-4 to bind SARS-CoV-2-S1. Further, one *in vitro* study observed no DPP-4–SARS-CoV-2-spike binding (Xi *et al.* 2020). DPP-4 was found to be increased in airway samples from patients with COVID-19 (Amati *et al.* 2020; Maremanda *et al.* 2020), and a meta-analysis reported that its inhibition was associated with lower COVID-19 mortality (Yang *et al.* 2021), though this idea faces some resistance (Noh *et al.* 2021). Overall, more studies are needed to determine DPP-4's role in SARS-CoV-2 entry.

Other molecules involved in endocytosis, like PIKfyve (Baranov *et al.* 2020) and TPC2 (Grimm & Tang 2020), may be useful targets to reduce SARS-CoV-2 entry into cells. HDL scavenger receptor B type 1 has been shown to facilitate ACE2-mediated SARS-CoV-2 entry (Wei *et al.* 2020). Additionally, as previously mentioned, SARS-CoV-2–sACE2 can enter cells via receptor-mediated endocytosis using AT1R and/or AVPR1B. The relevance and impact of these proteins on viral entry in islet cells requires investigation.

Mediators as Pharmacological Targets

Several therapeutic agents have been proposed and/or approved for treating COVID-19. Here, we highlight two examples that specifically target SARS-CoV-2 entry mediators, and we contrast their potential utility in limiting COVID-19 infection of pancreatic endocrine cells.

Meplazumab is a humanised anti-CD147 monoclonal antibody. *In vitro* studies demonstrated its efficacy in blocking SARS-CoV-2 replication in Vero E6 cells and SARS-CoV-2-pseudovirus entry in human T cells (Wang *et al.* 2020). Several clinical trials are underway to test its effectiveness against COVID-19 infection in humans. In one of these trials, meplazumab was found to reduce disease severity and accelerate recovery, especially in severe and critical COVID-19 cases (Bian *et al.* 2021). Given that CD147 is highly expressed in islet endocrine cells, meplazumab may prove useful in reducing entry of SARS-CoV-2 and its deleterious effects in the islet.

Another drug of interest is nafamostat mesylate, which exhibits anti-inflammatory and anticoagulant properties and is currently approved to treat pancreatitis. Nafamostat mesylate is a serine protease inhibitor active against TMPRSS2 (Hempel *et al.* 2021). In a screen of 24 FDA-approved drugs, it was found to exhibit the greatest antiviral efficacy against SARS-CoV-2 in a human lung cell line whose susceptibility to virus entry is largely TMPRSS2-dependent (Ko *et al.* 2021). Nafamostat mesylate has also been shown to reduce SARS-CoV-2 infection in mouse models (Li *et al.* 2021). Despite these promising pre-clinical data, results in humans with COVID-19 infection have been discouraging. For example, there was no significant difference in time to clinical improvement (Zhuravel *et al.* 2021) or in-hospital outcomes (Inokuchi *et al.* 2021) between hospitalised patients with COVID-19 treated with versus without nafamostat. Another study reported no evidence of anti-inflammatory, anticoagulant or antiviral activity with nafamostat (Quinn *et al.* 2022). In considering these human data together with evidence that TMPRSS2 expression is moderately low in islet α and β cells, nafamostat mesylate may not be a good candidate for reducing entry of SARS-CoV-2 and its deleterious effects in the islet.

Conclusions

SARS-CoV-2 infects pancreatic endocrine cells, including β cells. The canonical ACE2-TMPRSS2 pathway (Fig. 1) has been extensively researched in airway epithelia, but it remains to be fully understood in the islet. Given that islet ACE2 and TMPRSS2 expression is uncertain, investigation into alternative mediators, including their interactions with one another, is needed to better understand mechanisms underlying SARS-CoV-2 infection of islets.

Many of the mediators discussed exhibit moderate to high expressions in the islet. Given their islet expression patterns coupled with their role in other cells, we propose that furin, CTSL, GRP78, NRP1, CD147 and heparan sulphate are likely mediators of SARS-CoV-2 entry in the endocrine pancreas (Fig. 3). Data on CD147 suggest that it may be an alternative receptor for SARS-CoV-2 entry. GRP78 has been shown to mediate SARS-CoV-2 entry; however, more study is needed to deduce its dependence on other receptors. Furin, CTSL, NRP1 and heparan sulphate may assist ACE2 and/or the dominant SARS-CoV-2 entry receptor in the islet to increase viral entry but are unlikely to facilitate viral entry independently. For ADAM17, more work is needed to resolve whether its upregulation after viral entry promotes or hinders infection by subsequent virions. Lastly, while TMPRSS2 mediates viral entry in other cell types, it does not seem to be adequately expressed in β cells for this function.

With respect to islet ACE2, its induction by pro-inflammatory cytokines (and ER stress) may make it an important contributor to islet SARS-CoV-2 entry (Fig. 2), including exacerbation of infection via feed-forward mechanisms. To better understand SARS-CoV-2 entry in the context of cytokine and ER stress, a potential avenue for further study is the ACE2/ADAM17/GRP78 interplay. In Fig. 4, we propose a model for how ACE2, GRP78, ADAM17 and CD147 may interact under conditions of ER stress, including the inflammatory response that accompanies COVID-19.

It is worth noting that SARS-CoV-2 has been shown to infiltrate α cells, therefore more study is needed to understand how this may impact β-cell function, especially since α-to-β cell communication is required for maintaining normal insulin secretion and glycemia. Finally, SARS-CoV-2-induced islet dysfunction likely occurs independent of direct viral entry, perhaps from inflammation and fibrosis in the islet microenvironment; however, the literature would suggest this may be in addition to the direct route of viral invasion.

In sum, systematic investigation of candidate SARS-CoV-2 entry mediators in the endocrine pancreas may inform therapeutic strategies to protect islets from the deleterious effects of COVID-19. Such strategies may need to be continually adapted to keep up with new variants of SARS-CoV-2, whose viral entry mechanisms may differ from previous variants.

Acknowledgements

The authors thank Rebecca Hull-Meichle (VA Puget Sound Health Care System and University of Washington, Seattle, WA, USA) and Sarah Malik (University of Washington, Seattle, WA, USA) for assistance with literature searches, manuscript review, and valuable discussions regarding the content.

Funding

This work was supported by pilot funding to S.Z. from the United States Department of Veterans Affairs, as well as the National Institutes of Health (P30DK017047), and Seattle Institute for Biomedical and Clinical Research.

References

Åkerfeldt MC, Howes J, Chan JY, Stevens VA, Boubenna N, McGuire HM, King C, Biden TJ & Laybutt DR 2008 Cytokine-induced β-Cell death is independent of Endoplasmic Reticulum stress signaling. Diabetes 57 3034–3044. (doi:10.2337/db07-1802) [PubMed: 18591394]

Al-Aly Z, Xie Y & Bowe B 2021 High-dimensional characterization of post-acute sequelae of COVID-19. Nature 594 259–264. (doi:10.1038/s41586-021-03553-9) [PubMed: 33887749]

Allagnat F, Fukaya M, Nogueira TC, Delaroche D, Welsh N, Marselli L, Marchetti P, Haefliger JA, Eizirik DL & Cardozo AK 2012 C/EBP homologous protein contributes to cytokine-induced pro-inflammatory responses and apoptosis in β-cells. Cell Death and Differentiation 19 1836–1846. (doi:10.1038/cdd.2012.67) [PubMed: 22653339]

Amati F, Vancheri C, Latini A, Colona VL, Grelli S, D'Apice MR, Balestrieri E, Passarelli C, Minutolo A, Loddo S et al. 2020 Expression profiles of the SARS-CoV-2 host invasion genes in nasopharyngeal and oropharyngeal swabs of COVID-19 patients. Heliyon 6 e05143. (doi:10.1016/j.heliyon.2020.e05143) [PubMed: 33024851]

Anand P, Puranik A, Aravamudan M, Venkatakrishnan AJ & Soundararajan V 2020 SARS-CoV-2 strategically mimics proteolytic activation of human ENaC. ELife 9 e58603. (doi:10.7554/eLife.58603) [PubMed: 32452762]

Aoe T 2020 Pathological aspects of COVID-19 as a conformational disease and the use of pharmacological chaperones as a potential therapeutic strategy. Frontiers in Pharmacology 11 1095. (doi:10.3389/fphar.2020.01095) [PubMed: 32754041]

Baranov MV, Bianchi F & van den Bogaart G 2020 The PIKfyve inhibitor Apilimod: a double-edged sword against COVID-19. Cells 10 30. (doi:10.3390/cells10010030) [PubMed: 33375410]

Bian H, Zheng Z-H, Wei D, Wen A, Zhang Z, Lian J-Q, Kang W-Z, Hao C-Q, Wang J, Xie R-H et al. 2021 Safety and efficacy of meplazumab in healthy volunteers and COVID-19 patients: a randomized phase 1 and an exploratory phase 2 trial. Signal Transduction and Targeted Therapy 6 194. (doi:10.1038/s41392-021-00603-6) [PubMed: 34001849]

Brar GS, Barrow BM, Watson M, Griesbach R, Choung E, Welch A, Ruzsicska B, Raleigh DP & Zraika S 2017 Neprilysin is required for angiotensin-(1–7)'s ability to enhance insulin secretion via its proteolytic activity to generate angiotensin-(1–2). Diabetes 66 2201–2212. (doi:10.2337/db16-1318) [PubMed: 28559246]

Brouwers B, Coppola I, Vints K, Dislich B, Jouvet N, Van Lommel L, Segers C, Gounko NV, Thorrez L, Schuit F et al. 2021 Loss of furin in β cells induces an mTORC1-ATF4 anabolic pathway that leads to β-Cell dysfunction. Diabetes 70 492–503. (doi:10.2337/db20-0474) [PubMed: 33277337]

Brozzi F, Nardelli TR, Lopes M, Millard I, Barthson J, Igoillo-Esteve M, Grieco FA, Villate O, Oliveira JM, Casimir M et al. 2015 Cytokines induce endoplasmic reticulum stress in human, rat and mouse beta cells via different mechanisms. Diabetologia 58 2307–2316. (doi:10.1007/s00125-015-3669-6) [PubMed: 26099855]

Bugliani M, Syed F, Paula FMM, Omar BA, Suleiman M, Mossuto S, Grano F, Cardarelli F, Boggi U, Vistoli F et al. 2018 DPP-4 is expressed in human pancreatic beta cells and its direct inhibition improves beta cell function and survival in type 2 diabetes. Molecular and Cellular Endocrinology 473 186–193. (doi:10.1016/j.mce.2018.01.019) [PubMed: 29409957]

Cameron K, Rozano L, Falasca M & Mancera RL 2021 Does the SARS-CoV-2 spike protein receptor binding domain interact effectively with the DPP4 (CD26) receptor? A molecular docking study. International Journal of Molecular Sciences 22 7001. (doi:10.3390/ijms22137001) [PubMed: 34209788]

Cantuti-Castelvetri L, Ojha R, Pedro LD, Djannatian M, Franz J, Kuivanen S, van der Meer F, Kallio K, Kaya T, Anastasina M et al. 2020 Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. Science (New York, N.Y.) 370 856–860. (doi:10.1126/science.abd2985) [PubMed: 33082293]

Cardozo AK, Ortis F, Storling J, Feng Y-M, Rasschaert J, Tonnesen M, Van Eylen F, Mandrup-Poulsen T, Herchuelz A & Eizirik DL 2005 Cytokines Downregulate the Sarcoendoplasmic Reticulum Pump Ca2+ ATPase 2b and Deplete Endoplasmic Reticulum Ca2+, Leading to Induction of Endoplasmic Reticulum Stress in Pancreatic β-Cells. Diabetes 54 452 LP–461. (doi:10.2337/diabetes.54.2.452) [PubMed: 15677503]

Carlos AJ, Ha DP, Yeh D-W, Van Krieken R, Tseng C-C, Zhang P, Gill P, Machida K & Lee AS 2021 The chaperone GRP78 is a host auxiliary factor for SARS-CoV-2 and GRP78 depleting antibody blocks viral entry and infection. The Journal of Biological Chemistry 296 100759. (doi:10.1016/j.jbc.2021.100759) [PubMed: 33965375]

Chan C-P, Siu K-L, Chin K-T, Yuen K-Y, Zheng B, Jin D-Y, Ching-Ping C, Kam-Leung S, King-Tung C, Kwok-Yung Y et al. 2006 Modulation of the unfolded protein response by the severe acute respiratory syndrome coronavirus spike protein. Journal of Virology 80 9279–9287. (doi:10.1128/JVI.00659-06) [PubMed: 16940539]

Chen Y-W, Lee M-S, Lucht A, Chou F-P, Huang W, Havighurst TC, Kim K, Wang J-K, Antalis TM, Johnson MD et al. 2010 TMPRSS2, a serine protease expressed in the prostate on the apical surface of luminal epithelial cells and released into semen in prostasomes, is misregulated in prostate cancer cells. The American Journal of Pathology 176 2986–2996. (doi:10.2353/ajpath.2010.090665) [PubMed: 20382709]

Chu H, Chan C-M, Zhang X, Wang Y, Yuan S, Zhou J, Au-Yeung RK-H, Sze K-H, Yang D, Shuai H et al. 2018 Middle East respiratory syndrome coronavirus and bat coronavirus HKU9 both can utilize GRP78 for attachment onto host cells. The Journal of Biological Chemistry 293 11709–11726. (doi:10.1074/jbc.RA118.001897) [PubMed: 29887526]

Claas ECJ, Osterhaus ADME, van Beek R, De Jong JC, Rimmelzwaan GF, Senne DA, Krauss S, Shortridge KF & Webster RG 1998 Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. The Lancet 351 472–477. (doi:10.1016/S0140-6736(97)11212-0)

Clausen TM, Sandoval DR, Spliid CB, Pihl J, Perrett HR, Painter CD, Narayanan A, Majowicz SA, Kwong EM, McVicar RN et al. 2020 SARS-CoV-2 infection depends on cellular heparan sulfate and ACE2. Cell 183 1043–1057.e15. (doi:10.1016/j.cell.2020.09.033) [PubMed: 32970989]

Coate KC, Cha J, Shrestha S, Wang W, Gonçalves LM, Almaça J, Kapp ME, Fasolino M, Morgan A, Dai C et al. 2020 SARS-CoV-2 cell entry factors ACE2 and TMPRSS2 are expressed in the microvasculature and ducts of human pancreas but are not enriched in β Cells. Cell Metabolism 32 1028–1040.e4. (doi:10.1016/j.cmet.2020.11.006) [PubMed: 33207245]

Coutard B, Valle C, de Lamballerie X, Canard B, Seidah NG & Decroly E 2020 The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. Antiviral Research 176 104742. (doi:10.1016/j.antiviral.2020.104742) [PubMed: 32057769]

Daly JL, Simonetti B, Klein K, Chen K-E, Williamson MK, Antón-Plágaro C, Shoemark DK, Simón-Gracia L, Bauer M, Hollandi R et al. 2020 Neuropilin-1 is a host factor for SARS-CoV-2 infection. Science (New York, N.Y.) 370 861–865. (doi:10.1126/science.abd3072) [PubMed: 33082294]

Dana D & Pathak SK 2020 A review of small molecule inhibitors and functional probes of human cathepsin L. Molecules 25 698. (doi:10.3390/molecules25030698) [PubMed: 32041276]

Dhounchak S, Popp SK, Brown DJ, Laybutt DR, Biden TJ, Bornstein SR, Parish CR & Simeonovic CJ 2021 Heparan sulfate proteoglycans in beta cells provide a critical link between endoplasmic reticulum stress, oxidative stress and type 2 diabetes. PloS One 16 e0252607. (doi:10.1371/journal.pone.0252607) [PubMed: 34086738]

El-Huneidi W, Hamad M & Taneera J 2021 Expression of SARS-CoV-2 receptor “ACE2” in human pancreatic β cells: to be or not to be! Islets 1–9. (doi:10.1080/19382014.2021.1954458)

Elfiky AA 2020 SARS-CoV-2 spike-heat shock protein A5 (GRP78) recognition may be related to the immersed human coronaviruses. Frontiers in Pharmacology 11 577467. (doi:10.3389/fphar.2020.577467) [PubMed: 33362542]

Fenizia C, Galbiati S, Vanetti C, Vago R, Clerici M, Tacchetti C & Daniele T 2021 SARS-CoV-2 entry: at the crossroads of CD147 and ACE2. Cells 10 1434. (doi:10.3390/cells10061434) [PubMed: 34201214]

Fignani D, Licata G, Brusco N, Nigi L, Grieco GE, Marselli L, Overbergh L, Gysemans C, Colli ML, Marchetti P et al. 2020 SARS-CoV-2 receptor angiotensin I-converting enzyme type 2 (ACE2) is expressed in human pancreatic β-cells and in the human pancreas microvasculature. Frontiers in Endocrinology 11 876.

Gagnon ML, Bielenberg DR, Gechtman Z, Miao HQ, Takashima S, Soker S & Klagsbrun M 2000 Identification of a natural soluble neuropilin-1 that binds vascular endothelial growth factor: In vivo expression and antitumor activity. Proceedings of the National Academy of Sciences of the United States of America 97 2573–2578. (doi:10.1073/pnas.040337597) [PubMed: 10688880]

Geng J, Chen L, Yuan Y, Wang K, Wang Y, Qin C, Wu G, Chen R, Zhang Z, Wei D et al. 2021 CD147 antibody specifically and effectively inhibits infection and cytokine storm of SARS-CoV-2 and its variants delta, alpha, beta, and gamma. Signal Transduction and Targeted Therapy 6 347. (doi:10.1038/s41392-021-00760-8) [PubMed: 34564690]

Gomes CP, Fernandes DE, Casimiro F, da Mata GF, Passos MT, Varela P, Mastroianni-Kirsztajn G & Pesquero JB 2020 Cathepsin L in COVID-19: from pharmacological evidences to genetics. Frontiers in Cellular and Infection Microbiology 10 777. (doi:10.3389/fcimb.2020.589505)

Grass GD & Toole BP 2016 How, with whom and when: an overview of CD147-mediated regulatory networks influencing matrix metalloproteinase activity. Bioscience Reports 36 e00283. (doi:10.1042/BSR20150256)

Grimm C & Tang R 2020 Could an endo-lysosomal ion channel be the Achilles heel of SARS-CoV2? Cell Calcium 88 102212. (doi:10.1016/j.ceca.2020.102212) [PubMed: 32402856]

Guillot S, Delaval P, Brinchault G, Caulet-Maugendre S, Depince A, Lena H, Delatour B, Lagente V & Martin-Chouly C 2006 Increased extracellular matrix metalloproteinase inducer (EMMPRIN) expression in pulmonary fibrosis. Experimental Lung Research 32 81–97. (doi:10.1080/01902140600710512) [PubMed: 16754474]

Ha DP, Van Krieken R, Carlos AJ & Lee AS 2020 The stress-inducible molecular chaperone GRP78 as potential therapeutic target for coronavirus infection. The Journal of Infection 81 452–482. (doi:10.1016/j.jinf.2020.06.017)

Haga S, Yamamoto N, Nakai-Murakami C, Osawa Y, Tokunaga K, Sata T, Yamamoto N, Sasazuki T & Ishizaka Y 2008 Modulation of TNF-α-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TNF-α production and facilitates viral entry. Proceedings of the National Academy of Sciences 105 7809–7814. (doi:10.1073/pnas.0711241105)

Haga S, Nagata N, Okamura T, Yamamoto N, Sata T, Yamamoto N, Sasazuki T & Ishizaka Y 2010 TACE antagonists blocking ACE2 shedding caused by the spike protein of SARS-CoV are candidate antiviral compounds. Antiviral Research 85 551–555. (doi:10.1016/j.antiviral.2009.12.001) [PubMed: 19995578]

Hasan NM, Kendrick MA, Druckenbrod NR, Huelsmeyer MK, Warner TF & MacDonald MJ 2010 Genetic association of the neuropilin-1 gene with type 1 diabetes in children: Neuropilin-1 expression in pancreatic islets. Diabetes Research and Clinical Practice 87 e29–e32. (doi:10.1016/j.diabres.2009.12.016) [PubMed: 20053475]

Helal MA, Shouman S, Abdelwaly A, Elmehrath AO, Essawy M, Sayed SM, Saleh AH & El-Badri N 2020 Molecular basis of the potential interaction of SARS-CoV-2 spike protein to CD147 in COVID-19 associated-lymphopenia. Journal of Biomolecular Structure and Dynamics 1–11. (doi:10.1080/07391102.2020.1822208)

Hempel T, Raich L, Olsson S, Azouz NP, Klingler AM, Hoffmann M, Pöhlmann S, Rothenberg ME & Noé F 2021 Molecular mechanism of inhibiting the SARS-CoV-2 cell entry facilitator TMPRSS2 with camostat and nafamostat. Chemical Science 12 983–992. (doi:10.1039/D0SC05064D) [PubMed: 35382133]

Heurich A, Hofmann-Winkler H, Gierer S, Liepold T, Jahn O & Pöhlmann S 2014 TMPRSS2 and ADAM17 cleave ACE2 differentially and only proteolysis by TMPRSS2 augments entry driven by the severe acute respiratory syndrome coronavirus spike protein. Journal of Virology 88 1293–1307. (doi:10.1128/JVI.02202-13) [PubMed: 24227843]

Hikmet F, Méar L, Edvinsson Å, Micke P, Uhlén M & Lindskog C 2020 The protein expression profile of ACE2 in human tissues. Molecular Systems Biology 16 e9610. (doi:10.15252/msb.20209610) [PubMed: 32715618]

Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu N-H, Nitsche A et al. 2020a SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 181 271–280.e8. (doi:10.1016/j.cell.2020.02.052) [PubMed: 32142651]

Hoffmann M, Kleine-Weber H & Pöhlmann S 2020b A multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. Molecular Cell 78 779–784.e5. (doi:10.1016/j.molcel.2020.04.022) [PubMed: 32362314]

Hoffmann M, Hofmann-Winkler H, Smith JC, Krüger N, Arora P, Sørensen LK, Søgaard OS, Hasselstrøm JB, Winkler M, Hempel T et al. 2021 Camostat mesylate inhibits SARS-CoV-2 activation by TMPRSS2-related proteases and its metabolite GBPA exerts antiviral activity. EBioMedicine 65. (doi:10.1016/j.ebiom.2021.103255)

Huang Y, Yang C, Xu X, Xu W & Liu S 2020 Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacologica Sinica 41 1141–1149. (doi:10.1038/s41401-020-0485-4) [PubMed: 32747721]

Hull RL, Zraika S, Udayasankar J, Aston-Mourney K, Subramanian SL & Kahn SE 2009 Amyloid formation in human IAPP transgenic mouse islets and pancreas, and human pancreas, is not associated with endoplasmic reticulum stress. Diabetologia 52 1102–1111. (doi:10.1007/s00125-009-1329-4) [PubMed: 19352619]

Ibrahim IM, Abdelmalek DH & Elfiky AA 2019 GRP78: A cell's response to stress. Life Sciences 226 156–163. (doi:10.1016/j.lfs.2019.04.022) [PubMed: 30978349]

Ibrahim IM, Abdelmalek DH, Elshahat ME & Elfiky AA 2020 COVID-19 spike-host cell receptor GRP78 binding site prediction. The Journal of Infection 80 554–562. (doi:10.1016/j.jinf.2020.02.026) [PubMed: 32169481]

Inokuchi R, Kuno T, Komiyama J, Uda K, Miyamoto Y, Taniguchi Y, Abe T, Ishimaru M, Adomi M, Tamiya N et al. 2021 Association between nafamostat mesylate and in-hospital mortality in

patients with coronavirus disease 2019: a multicenter observational study. Journal of Clinical Medicine 11 116. (doi:10.3390/jcm11010116) [PubMed: 35011857]

Jaimes JA, Millet JK & Whittaker GR 2020 Proteolytic cleavage of the SARS-CoV-2 spike protein and the role of the novel S1/S2 site. IScience 23 101212. (doi:10.1016/j.isci.2020.101212) [PubMed: 32512386]

Katopodis P, Kerslake R, Davies J, Randeva SH, Chatha K, Hall M, Spandidos AD, Anikin V, Polychronis A, Robertus LJ et al. 2021 COVID-19 and SARS-CoV-2 host cell entry mediators: Expression profiling of TMRSS4 in health and disease. Int J Mol Med 47 64. (doi:10.3892/ijmm.2021.4897) [PubMed: 33649798]

Kayo T, Konda Y, Tanaka S, Takata K, Koizumi A & Takeuchi T 1996 Developmental expression of proprotein-processing endoprotease furin in rat pancreatic islets. Endocrinology 137 5126–5134. (doi:10.1210/endo.137.11.8895387) [PubMed: 8895387]

Ke X, Fei F, Chen Y, Xu L, Zhang Z, Huang Q, Zhang H, Yang H, Chen Z & Xing J 2012 Hypoxia upregulates CD147 through a combined effect of HIF-1α and Sp1 to promote glycolysis and tumor progression in epithelial solid tumors. Carcinogenesis 33 1598–1607. (doi:10.1093/carcin/bgs196) [PubMed: 22678117]

Ko M, Jeon S, Ryu W-S & Kim S 2021 Comparative analysis of antiviral efficacy of FDA-approved drugs against SARS-CoV-2 in human lung cells. Journal of Medical Virology 93 1403–1408. (doi:10.1002/jmv.26397) [PubMed: 32767684]

Köseler A, Sabirli R, Gören T, Türkçüer I & Kurt Ö 2020 Endoplasmic reticulum stress markers in SARS-COV-2 infection and pneumonia: case-control study. In Vivo 34 1645–1650. (doi:10.21873/invivo.11956) [PubMed: 32503824]

Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W et al. 2005 A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus–induced lung injury. Nature Medicine 11 875–879. (doi:10.1038/nm1267)

Kusmartseva I, Wu W, Syed F, Van Der Heide V, Jorgensen M, Joseph P, Tang X, Candelario-Jalil E, Yang C, Nick H et al. 2020 Expression of SARS-CoV-2 entry factors in the pancreas of normal organ donors and individuals with COVID-19. Cell Metabolism 32 1041–1051.e6. (doi:10.1016/j.cmet.2020.11.005) [PubMed: 33207244]

Kyrou I, Randeva HS, Spandidos DA & Karteris E 2021 Not only ACE2—the quest for additional host cell mediators of SARS-CoV-2 infection: Neuropilin-1 (NRP1) as a novel SARS-CoV-2 host cell entry mediator implicated in COVID-19. Signal Transduction and Targeted Therapy 6 21. (doi:10.1038/s41392-020-00460-9) [PubMed: 33462185]

Laybutt DR, Preston AM, Åkerfeldt MC, Kench JG, Busch AK, Biankin AV & Biden TJ 2007 Endoplasmic reticulum stress contributes to beta cell apoptosis in type 2 diabetes. Diabetologia 50 752–763. (doi:10.1007/s00125-006-0590-z) [PubMed: 17268797]

Lazartigues E, Qadir MMF & Mauvais-Jarvis F 2020 Endocrine significance of SARS-CoV-2's reliance on ACE2. Endocrinology 161 bqaa108. (doi:10.1210/endocr/bqaa108) [PubMed: 32652001]

Lee JJ, Kopetz S, Vilar E, Shen JP, Chen K & Maitra A 2020 Relative abundance of SARS-CoV-2 entry genes in the enterocytes of the lower gastrointestinal tract. Genes 11 645. (doi:10.3390/genes11060645) [PubMed: 32545271]

Li Z & Buck M 2021 Neuropilin-1 assists SARS-CoV-2 infection by stimulating the separation of Spike protein S1 and S2. Biophysical Journal 120 2828–2837. (doi:10.1016/j.bpj.2021.05.026) [PubMed: 34087218]

Li Y, Zhang Z, Yang L, Lian X, Xie Y, Li S, Xin S, Cao P & Lu J 2020 The MERS-CoV receptor DPP4 as a candidate binding target of the SARS-CoV-2 spike. IScience 23 101160. (doi:10.1016/j.isci.2020.101160) [PubMed: 32405622]

Li K, Meyerholz DK, Bartlett JA & McCray PBJ 2021 The TMPRSS2 inhibitor nafamostat reduces SARS-CoV-2 pulmonary infection in mouse models of COVID-19. MBio 12 e0097021. (doi:10.1128/mBio.00970-21) [PubMed: 34340553]

van Lier D, Kox M, Santos K, van der Hoeven H, Pillay J & Pickkers P 2021 Increased blood angiotensin converting enzyme 2 activity in critically ill COVID-19 patients. ERJ Open Research 7 00848–02020. (doi:10.1183/23120541.00848-2020) [PubMed: 33738305]

Liu F, Long X, Zhang B, Zhang W, Chen X & Zhang Z 2020 ACE2 expression in pancreas may cause pancreatic damage after SARS-CoV-2 infection. Clinical Gastroenterology and Hepatology : The Official Clinical Practice Journal of the American Gastroenterological Association 18 2128–2130.e2. (doi:10.1016/j.cgh.2020.04.040) [PubMed: 32334082]

Lo C-W, Kryvalap Y, Sheu T-J, Chang C-H & Czyzyk J 2019 Cellular proliferation in mouse and human pancreatic islets is regulated by serpin B13 inhibition and downstream targeting of E-cadherin by cathepsin L. Diabetologia 62 822–834. (doi:10.1007/s00125-019-4834-0) [PubMed: 30824970]

Louagie E, Taylor NA, Flamez D, Roebroek AJM, Bright NA, Meulemans S, Quintens R, Herrera PL, Schuit F, Van de Ven WJM et al. 2008 Role of furin in granular acidification in the endocrine pancreas: identification of the V-ATPase subunit Ac45 as a candidate substrate. Proceedings of the National Academy of Sciences of the United States of America 105 12319–12324. (doi:10.1073/pnas.0800340105) [PubMed: 18713856]

Maehr R, Mintern JD, Herman AE, Lennon-Duménil A-M, Mathis D, Benoist C & Ploegh HL 2005 Cathepsin L is essential for onset of autoimmune diabetes in NOD mice. The Journal of Clinical Investigation 115 2934–2943. (doi:10.1172/JCI25485) [PubMed: 16184198]

Marchetti P, Bugliani M, Lupi R, Marselli L, Masini M, Boggi U, Filipponi F, Weir GC, Eizirik DL & Cnop M 2007 The endoplasmic reticulum in pancreatic beta cells of type 2 diabetes patients. Diabetologia 50 2486–2494. (doi:10.1007/s00125-007-0816-8) [PubMed: 17906960]

Maremanda KP, Sundar IK, Li D & Rahman I 2020 Age-dependent assessment of genes involved in cellular senescence, telomere, and mitochondrial pathways in human lung tissue of smokers, COPD, and IPF: associations with SARS-CoV-2 COVID-19 ACE2-TMPRSS2-Furin-DPP4 Axis. Frontiers in Pharmacology 11 1356. (doi:10.3389/fphar.2020.584637)

Marselli L, Piron A, Suleiman M, Colli ML, Yi X, Khamis A, Carrat GR, Rutter GA, Bugliani M, Giusti L et al. 2020 Persistent or transient human β cell dysfunction induced by metabolic stress: specific signatures and shared gene expression with type 2 diabetes. Cell Reports 33 108466. (doi:10.1016/j.celrep.2020.108466) [PubMed: 33264613]

Matsuyama S, Ujike M, Morikawa S, Tashiro M & Taguchi F 2005 Protease-mediated enhancement of severe acute respiratory syndrome coronavirus infection. Proceedings of the National Academy of Sciences of the United States of America 102 12543–12547. (doi:10.1073/pnas.0503203102) [PubMed: 16116101]

Mine K, Nagafuchi S, Mori H, Takahashi H & Anzai K 2022 SARS-CoV-2 infection and pancreatic beta-cell failure. Biology 11 22. (doi:10.3390/biology11010022)

Misra S, Barron E, Vamos E, Thomas S, Dhatariya K, Kar P, Young B, Khunti K & Valabhji J 2021 Temporal trends in emergency admissions for diabetic ketoacidosis in people with diabetes in England before and during the COVID-19 pandemic: a population-based study. The Lancet. Diabetes & Endocrinology 9 671–680. (doi:10.1016/S2213-8587(21)00208-4) [PubMed: 34481558]

Müller JA, Groß R, Conzelmann C, Krüger J, Merle U, Steinhart J, Weil T, Koepke L, Bozzo CP, Read C et al. 2021 SARS-CoV-2 infects and replicates in cells of the human endocrine and exocrine pancreas. Nature Metabolism 3 149–165. (doi:10.1038/s42255-021-00347-1)

Murgolo N, Therien AG, Howell B, Klein D, Koeplinger K, Lieberman LA, Adam GC, Flynn J, McKenna P, Swaminathan G et al. 2021 SARS-CoV-2 tropism, entry, replication, and propagation: Considerations for drug discovery and development. PLOS Pathogens 17 e1009225. [PubMed: 33596266]

Mycroft-West CJ, Su D, Pagani I, Rudd TR, Elli S, Gandhi NS, Guimond SE, Miller GJ, Meneghetti MCZ, Nader HB et al. 2020 Heparin inhibits cellular invasion by SARS-CoV-2: structural dependence of the interaction of the spike S1 receptor-binding domain with heparin. Thrombosis and Haemostasis 120 1700–1715. (doi:10.1055/s-0040-1721319) [PubMed: 33368089]

Ni M, Zhang Y & Lee AS 2011 Beyond the endoplasmic reticulum: atypical GRP78 in cell viability, signalling and therapeutic targeting. The Biochemical Journal 434 181–188. (doi:10.1042/BJ20101569) [PubMed: 21309747]

Noh Y, Oh I-S, Jeong HE, Filion KB, Yu OHY & Shin J-Y 2021 Association between DPP-4 inhibitors and COVID-19–related outcomes among patients with type 2 diabetes. Diabetes Care 44 e64–e66. (doi:10.2337/dc20-1824) [PubMed: 33547204]

Omar BA, Liehua L, Yamada Y, Seino Y, Marchetti P & Ahrén B 2014 Dipeptidyl peptidase 4 (DPP-4) is expressed in mouse and human islets and its activity is decreased in human islets from individuals with type 2 diabetes. Diabetologia 57 1876–1883. (doi:10.1007/s00125-014-3299-4) [PubMed: 24939431]

Onabajo OO, Banday AR, Stanifer ML, Yan W, Obajemu A, Santer DM, Florez-Vargas O, Piontkivska H, Vargas JM, Ring TJ et al. 2020 Interferons and viruses induce a novel truncated ACE2 isoform and not the full-length SARS-CoV-2 receptor. Nature Genetics 52 1283–1293. (doi:10.1038/s41588-020-00731-9) [PubMed: 33077916]

Padmanabhan P, Desikan R & Dixit NM 2020 Targeting TMPRSS2 and Cathepsin B/L together may be synergistic against SARS-CoV-2 infection. PLoS Computational Biology 16 e1008461. (doi:10.1371/journal.pcbi.1008461) [PubMed: 33290397]

Palacios Y, Ruiz A, Ramón-Luing LA, Ocaña-Guzman R, Barreto-Rodriguez O, Sánchez-Monciváis A, Tecuatzi-Cadena B, Regalado-García AG, Pineda-Gudiño RD, García-Martínez A et al. 2021 Severe COVID-19 patients show an increase in soluble TNFR1 and ADAM17, with a relationship to mortality. International Journal of Molecular Sciences 22 8423. (doi:10.3390/ijms22168423) [PubMed: 34445140]

Papa G, Mallery DL, Albecka A, Welch LG, Cattin-Ortolá J, Luptak J, Paul D, McMahon HT, Goodfellow IG, Carter A et al. 2021 Furin cleavage of SARS-CoV-2 Spike promotes but is not essential for infection and cell-cell fusion. PLOS Pathogens 17 e1009246. [PubMed: 33493182]

Peacock TP, Goldhill DH, Zhou J, Baillon L, Frise R, Swann OC, Kugathasan R, Penn R, Brown JC, Sanchez-David RY et al. 2021 The furin cleavage site in the SARS-CoV-2 spike protein is required for transmission in ferrets. Nature Microbiology 6 899–909. (doi:10.1038/s41564-021-00908-w)

Pellet-Many C, Frankel P, Jia H & Zachary I 2008 Neuropilins: structure, function and role in disease. Biochemical Journal 411 211–226. (doi:10.1042/BJ20071639) [PubMed: 18363553]

Pirot P, Eizirik DL & Cardozo AK 2006 Interferon-γ potentiates endoplasmic reticulum stress-induced death by reducing pancreatic beta cell defence mechanisms. Diabetologia 49 1229. (doi:10.1007/s00125-006-0214-7) [PubMed: 16604358]

Qadir MMF, Bhondeley M, Beatty W, Gaupp DD, Doyle-Meyers LA, Fischer T, Bandyopadhyay I, Blair RV, Bohm R, Rappaport J et al. 2021 SARS-CoV-2 infection of the pancreas promotes thrombofibrosis and is associated with new-onset diabetes. JCI Insight 6 e151551. (doi:10.1172/jci.insight.151551) [PubMed: 34241597]

Quinn TM, Gaughan EE, Bruce A, Antonelli J, O'Connor R, Li F, McNamara S, Koch O, MacKintosh C, Dockrell D et al. 2022 Randomised controlled trial of intravenous nafamostat mesylate in COVID pneumonitis: Phase 1b/2a experimental study to investigate safety, Pharmacokinetics and Pharmacodynamics. EBioMedicine 76 103856. (doi:10.1016/j.ebiom.2022.103856) [PubMed: 35152152]

Ragotte RJ, Pulido D, Donnellan FR, Hill ML, Gorini G, Davies H, Brun J, McHugh K, King LDW, Skinner K et al. 2021 Human basigin (CD147) does not directly interact with SARS-CoV-2 spike glycoprotein. MSphere 6 e0064721–e0064721. (doi:10.1128/mSphere.00647-21) [PubMed: 34378982]

Rosa-Fernandes L, Lazari LC, da Silva JM, de Morais Gomes V, Machado RRG, dos Santos AF, Araujo DB, Coutinho JVP, Arini GS, Angeli CB et al. 2021 SARS-CoV-2 activates ER stress and unfolded protein response. BioRxiv 2021.06.21.449284. (doi:10.1101/2021.06.21.449284)

Rzymski T, Petry A, Kračun D, Rieß F, Pike L, Harris AL & Görlach A 2012 The unfolded protein response controls induction and activation of ADAM17/TACE by severe hypoxia and ER stress. Oncogene 31 3621–3634. (doi:10.1038/onc.2011.522) [PubMed: 22105359]

Sabirli R, Koseler A, Goren T, Turkcuer I & Kurt O 2021 High GRP78 levels in Covid-19 infection: A case-control study. Life Sciences 265 118781. (doi:10.1016/j.lfs.2020.118781) [PubMed: 33220289]

Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, Colagiuri S, Guariguata L, Motala AA, Ogurtsova K et al. 2019 Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9(th) edition. Diabetes Research and Clinical Practice 157 107843. (doi:10.1016/j.diabres.2019.107843) [PubMed: 31518657]

Sawada Y, Kameya T, Aizama T, Izumi T & Takeuchi T 2000 Proprotein-processing endoprotease furin and its substrate parathyroid hormone-related protein are coexpressed in insulinoma cells. Endocrine Pathology 11 31–39. (doi:10.1385/ep:11:1:31) [PubMed: 12114655]

Schäfer M, Granato DC, Krossa S, Bartels A-K, Yokoo S, Düsterhöft S, Koudelka T, Scheidig AJ, Tholey A, Paes Leme AF et al. 2017 GRP78 protects a disintegrin and metalloprotease 17 against protein-disulfide isomerase A6 catalyzed inactivation. FEBS Letters 591 3567–3587. (doi:10.1002/1873-3468.12858) [PubMed: 28949004]

Schreiber B, Patel A & Verma A 2021 Shedding light on COVID-19: ADAM17 the missing link? American Journal of Therapeutics 28.

Segerstolpe Å, Palasantza A, Eliasson P, Andersson E-M, Andréasson A-C, Sun X, Picelli S, Sabirsh A, Clausen M, Bjursell MK et al. 2016 Single-cell transcriptome profiling of human pancreatic islets in health and type 2 diabetes. Cell Metabolism 24 593–607. (doi:10.1016/j.cmet.2016.08.020) [PubMed: 27667667]

Shang J, Wan Y, Luo C, Ye G, Geng Q, Auerbach A & Li F 2020 Cell entry mechanisms of SARS-CoV-2. Proceedings of the National Academy of Sciences of the United States of America 117 11727–11734. (doi:10.1073/pnas.2003138117) [PubMed: 32376634]

Shapiro J, Sciaky N, Lee J, Bosshart H, Angeletti RH & Bonifacino JS 1997 Localization of endogenous furin in cultured cell lines. The Journal of Histochemistry and Cytochemistry : Official Journal of the Histochemistry Society 45 3–12. (doi:10.1177/002215549704500102) [PubMed: 9010463]

Shilts J, Crozier TWM, Greenwood EJD, Lehner PJ & Wright GJ 2021 No evidence for basigin/CD147 as a direct SARS-CoV-2 spike binding receptor. Scientific Reports 11 413. (doi:10.1038/s41598-020-80464-1) [PubMed: 33432067]

Steenblock C, Richter S, Berger I, Barovic M, Schmid J, Schubert U, Jarzebska N, von Mässenhausen A, Linkermann A, Schürmann A et al. 2021 Viral infiltration of pancreatic islets in patients with COVID-19. Nature Communications 12 3534. (doi:10.1038/s41467-021-23886-3)

Takahashi I, Noguchi N, Nata K, Yamada S, Kaneiwa T, Mizumoto S, Ikeda T, Sugihara K, Asano M, Yoshikawa T et al. 2009 Important role of heparan sulfate in postnatal islet growth and insulin secretion. Biochemical and Biophysical Research Communications 383 113–118. (doi:10.1016/j.bbrc.2009.03.140) [PubMed: 19336225]

Taneera J, El-Huneidi W, Hamad M, Mohammed AK, Elaraby E & Hachim MY 2020 Expression profile of SARS-CoV-2 host receptors in human pancreatic islets revealed upregulation of ACE2 in diabetic donors. Biology 9 215. (doi:10.3390/biology9080215) [PubMed: 32784802]

Tang J, Guo Y-S, Zhang Y, Yu X-L, Li L, Huang W, Li Y, Chen B, Jiang J-L & Chen Z-N 2012 CD147 induces UPR to inhibit apoptosis and chemosensitivity by increasing the transcription of Bip in hepatocellular carcinoma. Cell Death & Differentiation 19 1779–1790. (doi:10.1038/cdd.2012.60) [PubMed: 22595757]

Tang X, Yang M, Duan Z, Liao Z, Liu L, Cheng R, Fang M, Wang G, Liu H, Xu J et al. 2020 Transferrin receptor is another receptor for SARS-CoV-2 entry. BioRxiv 2020.10.23.350348. (doi:10.1101/2020.10.23.350348)

Tang X, Uhl S, Zhang T, Xue D, Li B, Vandana JJ, Acklin JA, Bonnycastle LL, Narisu N, Erdos MR et al. 2021a SARS-CoV-2 infection induces beta cell transdifferentiation. Cell Metabolism 33 1577–1591.e7. (doi:10.1016/j.cmet.2021.05.015) [PubMed: 34081913]

Tang T, Jaimes JA, Bidon MK, Straus MR, Daniel S & Whittaker GR 2021b Proteolytic activation of SARS-CoV-2 Spike at the S1/S2 Boundary: potential role of proteases beyond furin. ACS Infectious Diseases 7 264–272. (doi:10.1021/acsinfecdis.0c00701) [PubMed: 33432808]

Telenti A, Arvin A, Corey L, Corti D, Diamond MS, García-Sastre A, Garry RF, Holmes EC, Pang PS & Virgin HW 2021 After the pandemic: perspectives on the future trajectory of COVID-19. Nature 596 495–504. (doi:10.1038/s41586-021-03792-w) [PubMed: 34237771]

Thomas G 2002 Furin at the cutting edge: from protein traffic to embryogenesis and disease. Nature Reviews. Molecular Cell Biology 3 753–766. (doi:10.1038/nrm934) [PubMed: 12360192]

Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson Å, Kampf C, Sjöstedt E, Asplund A et al. 2015 Proteomics. Tissue-based map of the human proteome. Science (New York, N.Y.) 347 1260419. (doi:10.1126/science.1260419) [PubMed: 25613900]

Ulrich H & Pillat MM 2020 CD147 as a target for COVID-19 treatment: suggested effects of azithromycin and stem cell engagement. Stem Cell Reviews and Reports 16 434–440. (doi:10.1007/s12015-020-09976-7) [PubMed: 32307653]

Vankadari N & Wilce JA 2020 Emerging COVID-19 coronavirus: glycan shield and structure prediction of spike glycoprotein and its interaction with human CD26. Emerging Microbes & Infections 9 601–604. (doi:10.1080/22221751.2020.1739565) [PubMed: 32178593]

Vellanki P & Umpierrez GE 2017 Diabetic ketoacidosis: a common debut of diabetes among African Americans with type 2 diabetes. Endocrine Practice 23 971–978. (doi:10.4158/EP161679.RA) [PubMed: 28534682]

Wander PL, Lowy E, Beste LA, Tulloch-Palomino L, Korpak A, Peterson AC, Young BA & Boyko EJ 2021 Risk factors for adverse outcomes among 35 879 veterans with and without diabetes after diagnosis with COVID-19. BMJ Open Diabetes Research & Care 9 e002252. (doi:10.1136/bmjdrc-2021-002252)

Wander PL, Lowy E, Beste LA, Tulloch-Palomino L, Korpak A, Peterson AC, Kahn SE & Boyko EJ 2022 The incidence of diabetes among 2,777,768 veterans with and without recent SARS-CoV-2 infection. Diabetes Care 45 782–788. (doi:10.2337/dc21-1686) [PubMed: 35085391]

Wang M, Wang P, Peng J-L, Wu S, Zhao X-P, Li L & Shen G-X 2009 The altered expression of glucose-regulated proteins 78 in different phase of streptozotocin-affected pancreatic beta-cells. Cell Stress & Chaperones 14 43–48. (doi:10.1007/s12192-008-0053-1) [PubMed: 18597185]

Wang K, Chen W, Zhang Z, Deng Y, Lian J-Q, Du P, Wei D, Zhang Y, Sun X-X, Gong L et al. 2020 CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. Signal Transduction and Targeted Therapy 5 283. (doi:10.1038/s41392-020-00426-x) [PubMed: 33277466]

Wei C, Wan L, Yan Q, Wang X, Zhang J, Yang X, Zhang Y, Fan C, Li D, Deng Y et al. 2020 HDL-scavenger receptor B type 1 facilitates SARS-CoV-2 entry. Nature Metabolism 2 1391–1400. (doi:10.1038/s42255-020-00324-0)

Wruck W & Adjaye J 2020 SARS-CoV-2 receptor ACE2 is co-expressed with genes related to transmembrane serine proteases, viral entry, immunity and cellular stress. Scientific Reports 10 21415. (doi:10.1038/s41598-020-78402-2) [PubMed: 33293627]

Wu C-T, Lidsky PV, Xiao Y, Lee IT, Cheng R, Nakayama T, Jiang S, Demeter J, Bevacqua RJ, Chang CA et al. 2021 SARS-CoV-2 infects human pancreatic β cells and elicits β cell impairment. Cell Metabolism 33 1565–1576.e5. (doi:10.1016/j.cmet.2021.05.013) [PubMed: 34081912]

Xi CR, Di Fazio A, Nadvi NA, Patel K, Xiang MS, Zhang HE, Deshpande C, Low JK, Wang XT, Chen Y et al. 2020 A novel purification procedure for active recombinant human DPP4 and the inability of DPP4 to bind SARS-CoV-2. Molecules 25 5392. (doi:10.3390/molecules25225392) [PubMed: 33218025]

Xie Y & Al-Aly Z 2022 Risks and burdens of incident diabetes in long COVID: a cohort study. The Lancet. Diabetes & Endocrinology. (doi:10.1016/S2213-8587(22)00044-4)

Yadati T, Houben T, Bitorina A & Shiri-Sverdlov R 2020 The ins and outs of cathepsins: physiological function and role in disease management. Cells 9 1679. (doi:10.3390/cells9071679) [PubMed: 32668602]

Yang J-K, Lin S-S, Ji X-J & Guo L-M 2010 Binding of SARS coronavirus to its receptor damages islets and causes acute diabetes. Acta Diabetologica 47 193–199. (doi:10.1007/s00592-009-0109-4) [PubMed: 19333547]

Yang L, Han Y, Nilsson-Payant BE, Gupta V, Wang P, Duan X, Tang X, Zhu J, Zhao Z, Jaffré F et al. 2020 A human pluripotent stem cell-based platform to study SARS-CoV-2 tropism and model virus infection in human cells and organoids. Cell Stem Cell 27 125–136.e7. (doi:10.1016/j.stem.2020.06.015) [PubMed: 32579880]

Yang Y, Cai Z & Zhang J 2021 DPP-4 inhibitors may improve the mortality of coronavirus disease 2019: A meta-analysis. PLOS ONE 16 e0251916. [PubMed: 34015003]

Yeung ML, Teng JLL, Jia L, Zhang C, Huang C, Cai J-P, Zhou R, Chan K-H, Zhao H, Zhu L et al. 2021 Soluble ACE2-mediated cell entry of SARS-CoV-2 via interaction with proteins related to the renin-angiotensin system. Cell 184 2212–2228.e12. (doi:10.1016/j.cell.2021.02.053) [PubMed: 33713620]

Zang R, Castro MFG, McCune BT, Zeng Q, Rothlauf PW, Sonnek NM, Liu Z, Brulois KF, Wang X, Greenberg HB et al. 2020 TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. Science Immunology 5 eabc3582. (doi:10.1126/sciimmunol.abc3582) [PubMed: 32404436]

Zhang Q, Chen CZ, Swaroop M, Xu M, Wang L, Lee J, Wang AQ, Pradhan M, Hagen N, Chen L et al. 2020 Heparan sulfate assists SARS-CoV-2 in cell entry and can be targeted by approved drugs in vitro. Cell Discovery 6 80. (doi:10.1038/s41421-020-00222-5) [PubMed: 33298900]

Zhao C, Wilson MC, Schuit F, Halestrap AP & Rutter GA 2001 Expression and distribution of lactate/monocarboxylate transporter isoforms in pancreatic islets and the exocrine pancreas. Diabetes 50 361–366. (doi:10.2337/diabetes.50.2.361) [PubMed: 11272148]

Zhao M-M, Yang W-L, Yang F-Y, Zhang L, Huang W-J, Hou W, Fan C-F, Jin R-H, Feng Y-M, Wang Y-C et al. 2021 Cathepsin L plays a key role in SARS-CoV-2 infection in humans and humanized mice and is a promising target for new drug development. Signal Transduction and Targeted Therapy 6 134. (doi:10.1038/s41392-021-00558-8) [PubMed: 33774649]

Zhu Y, Feng F, Hu G, Wang Y, Yu Y, Zhu Y, Xu W, Cai X, Sun Z, Han W et al. 2021 A genome-wide CRISPR screen identifies host factors that regulate SARS-CoV-2 entry. Nature Communications 12 961. (doi:10.1038/s41467-021-21213-4)

Zhuravel SV, Khmelnitskiy OK, Burlaka OO, Gritsan AI, Goloshchekin BM, Kim S & Hong KY 2021 Nafamostat in hospitalized patients with moderate to severe COVID-19 pneumonia: a randomised Phase II clinical trial. EClinicalMedicine 41 101169. (doi:10.1016/j.eclinm.2021.101169) [PubMed: 34723164]

Ziolkowski AF, Popp SK, Freeman C, Parish CR & Simeonovic CJ 2012 Heparan sulfate and heparanase play key roles in mouse β cell survival and autoimmune diabetes. The Journal of Clinical Investigation 122 132–141. (doi:10.1172/JCI46177) [PubMed: 22182841]

Zipeto D, Palmeira J da F, Argañaraz GA & Argañaraz ER 2020 ACE2/ADAM17/TMPRSS2 interplay may be the main risk factor for COVID-19. Frontiers in Immunology 11 2642. (doi:10.3389/fimmu.2020.576745)

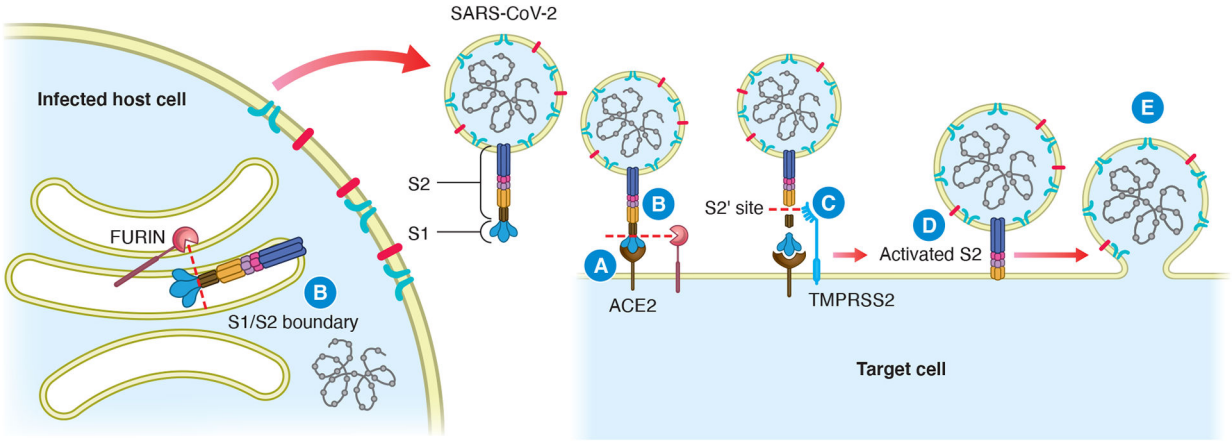

Figure 1:

ACE2-mediated SARS-CoV-2 viral entry via the membrane fusion pathway. SARS-CoV-2-spike binds to ACE2 on the target cell surface (step A), after which furin cleaves SARS-CoV-2-spike at the S1/S2 boundary (step B). Furin may also cleave the spike protein at the S1/S2 boundary during viral production, prior to virus release into the extracellular space. TMPRSS2 cleaves the S2 protein at the S2’ site (step C), which allows the insertion of hydrophobic amino acid residues in the activated S2 subunit into the plasma membrane (step D), facilitating membrane fusion between the viral envelope and the host cell plasma membrane (step E).

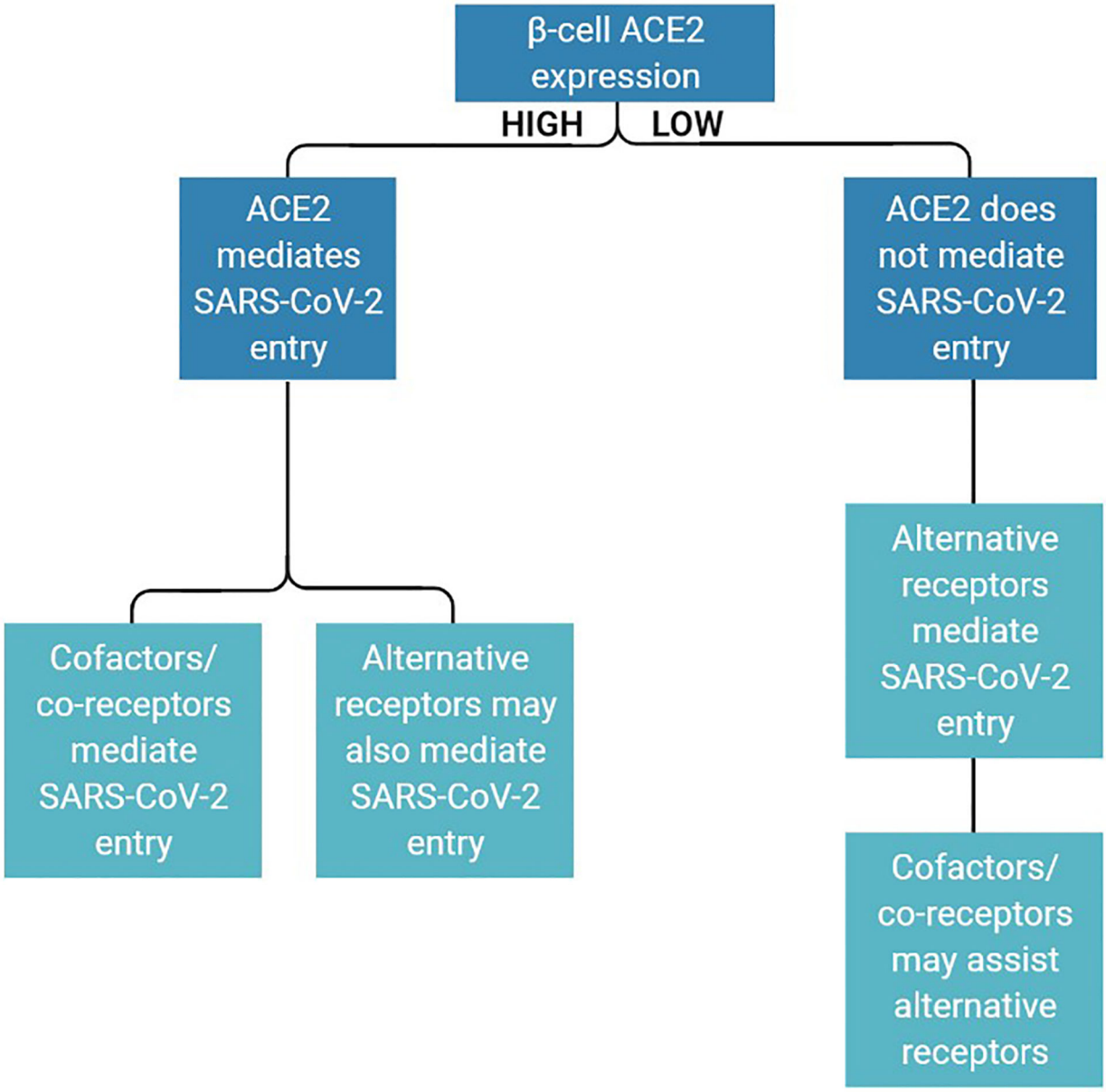

Figure 2:

Potential involvement of SARS-CoV-2 entry factors in islet endocrine cells under conditions of high or low ACE2 expression. ACE2 serves as the predominant SARS-CoV-2 receptor when its levels are sufficiently high for viral entry, and/or its expression is induced by pro-inflammatory cytokines. Under these circumstances, cofactors/co-receptors assist ACE2, and co-receptors further allow viral entry. If ACE2 levels are too low to permit SARS-CoV-2 entry, alternative receptors facilitate viral entry, with assistance from cofactors/co-receptors.

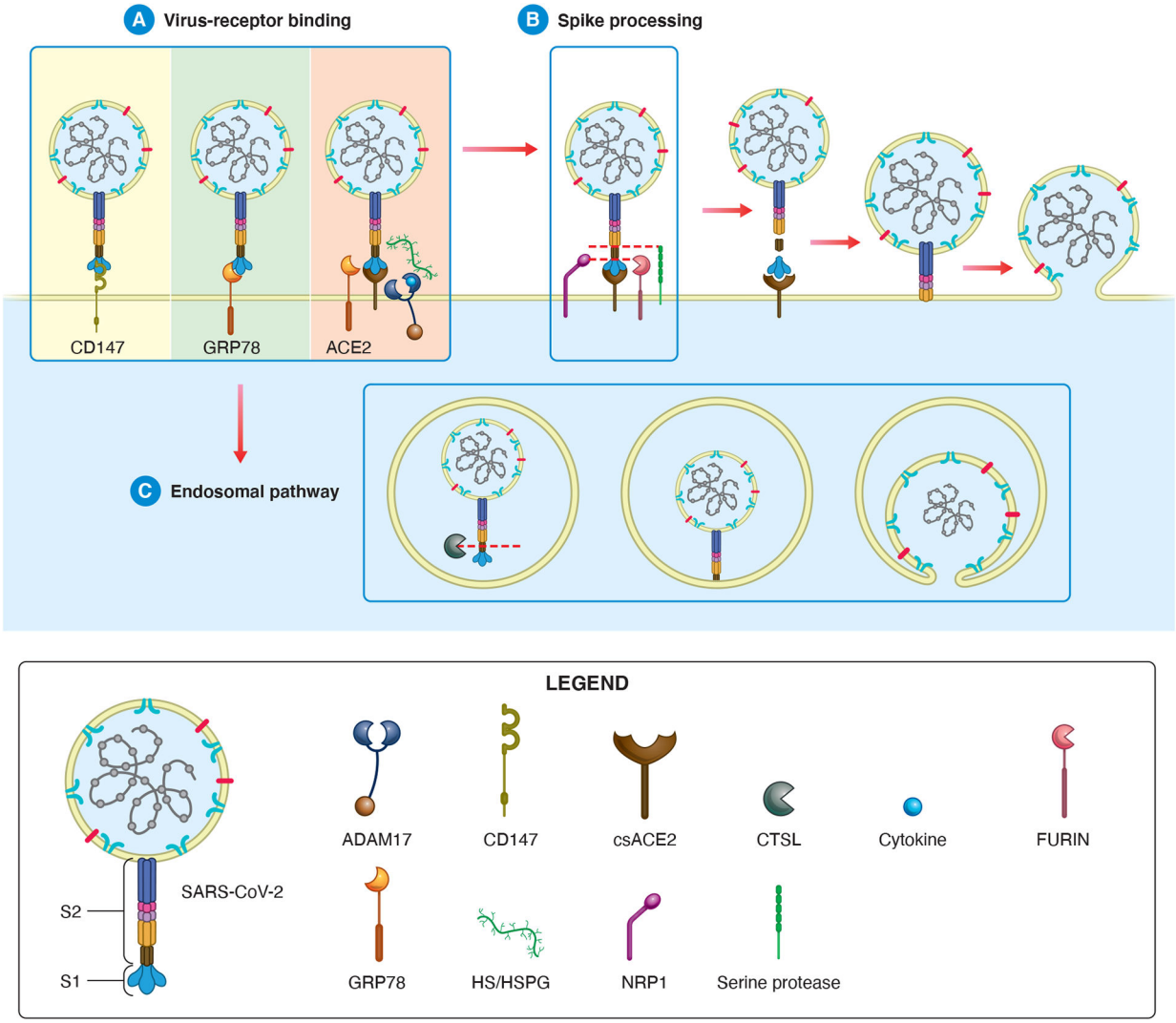

Figure 3:

Proposed roles for alternative receptors and mediators of viral entry into β cells based on studies in other cell types. (A) The mature virion binds to its host cell receptor, which may be CD147 (left), GRP78 (centre) or ACE2 (right). If the host cell receptor is ACE2, SARS-CoV-2-spike-ACE2 binding may be facilitated by GRP78 or HS/HSPG. ADAM17 may be involved in ACE2-mediated SARS-CoV-2 entry in several ways. On one hand, ADAM17 cleaves csACE2, releasing sACE2, which can impede SARS-CoV-2-receptor binding. On the other hand, ADAM17 can cleave and activate cytokines for release, which may upregulate viral entry mediators. On the whole, the net impact of ADAM17 on viral entry is unclear. (B) Before membrane fusion, the spike protein must be processed. After S1/S2 cleavage by furin (which can also occur during viral production), NRP1 can stabilise the SARS-Cov-2-S1-CendR motif to increase the rate of viral spike processing. S2' cleavage may occur by another host protease in the absence of TMPRSS2. (C) Alternatively, rather than viral envelope-host cell membrane fusion, the SARS-CoV-2-receptor complex can be taken up via receptor-mediated endocytosis. CTSL in the endosome cleaves SARS-CoV-2-

spike at a site distinct from the S1/S2 boundary and S2’ site. Viral entry is complete when the viral envelope fuses with the host cell surface membrane or endosomal membrane.

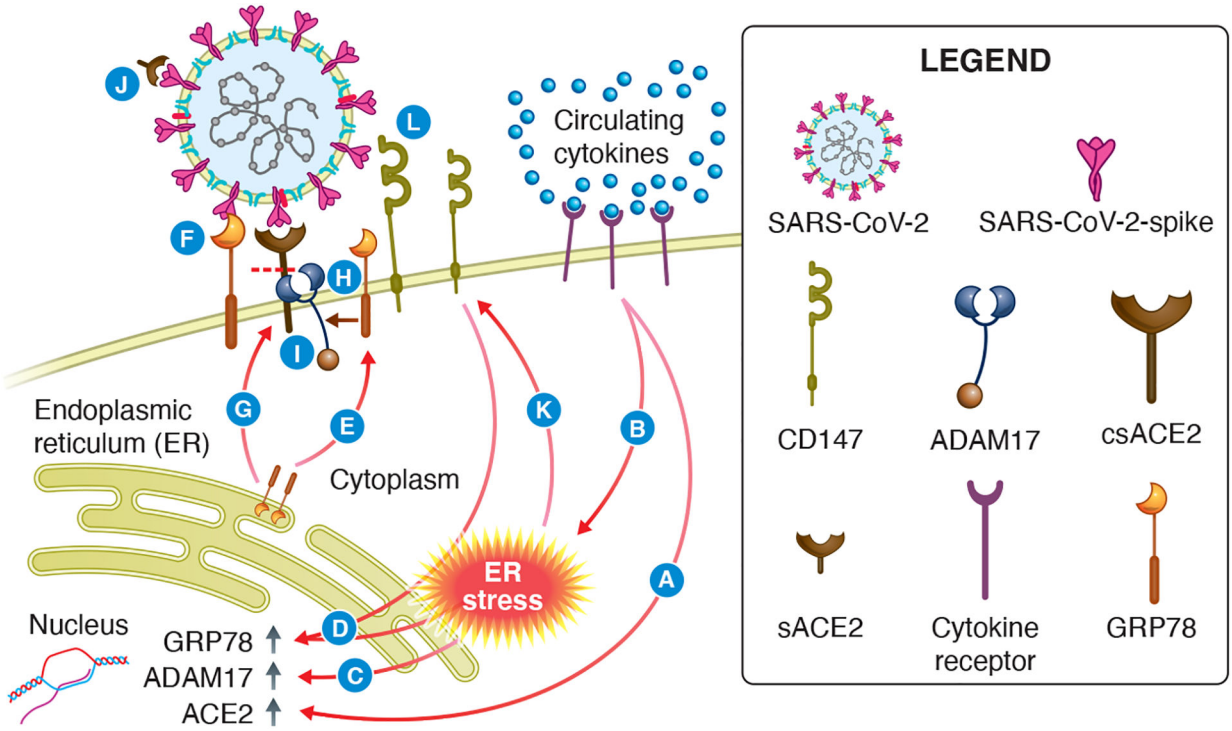

Figure 4:

Hypothesized interaction of ACE2, ADAM17, CD147 and GRP78 under conditions of elevated pro-inflammatory cytokines and ER stress. In patients with COVID-19, pancreatic endocrine cells are bathed in cytokines derived from the circulation. This state (A) upregulates ACE2 gene expression and (B) ER stress, which causes (C) an increase in ADAM17 mRNA levels. (D) ER stress also increases GRP78 mRNA levels, mediated by CD147. The subsequent increase in GRP78 protein results in (E) missorting and translocation of GRP78 to the cell surface, where it may (F) act as an alternative receptor or ACE2 cofactor during SARS-CoV-2 entry. (G) GRP78 is also involved in ACE2 trafficking to the plasma membrane. Additionally, GRP78 (H) protects ADAM17 from inhibition, which allows ADAM17 to (I) cleave plasma membrane bound ACE2 at an increased rate, thereby elevating soluble ACE2 levels, which may (J) bind to the spike protein and inhibit SARS-CoV-2 entry. At the same time, (K) ER stress upregulates CD147. The consequent increase in CD147 at the cell surface can (L) increase CD147-mediated SARS-CoV-2 entry.