SARS-CoV-2 infection: the lock and the key

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Note: highlighted in italic are information for non-biologists

Introduction

All viral infections require the interaction of viral particles with the surface of the host cells. We have to imagine our cells to possess on their membrane a number of locks, called receptors, each one holding, usually, a specific self key, named ligand, which is a protein, either soluble or present on the surface of neighbouring cells. When the key enters into the lock, other proteins are activated inside the cells, working in sequence and constituting a signaling cascade up to the nucleus. This cascade of events leads to changes in the expression of genes which has to be appropriate to the signal the cell has received. To make things as clear as possible, it is like the activation of a car engine, where the cell is the car and the engine the nucleus, which drives cell behavior. An example is the response to proteins (e.g. growth factors) which indicate to the cell it has to proliferate and divide in two daughter cells. Viruses repeatedly evolve some of their surface proteins to use these locks to enter into host cells, particularly when they have to jump from a species to another one, in order to make lock-key interaction stronger. This is also the case of CoV-2 Spike protein (the key) which

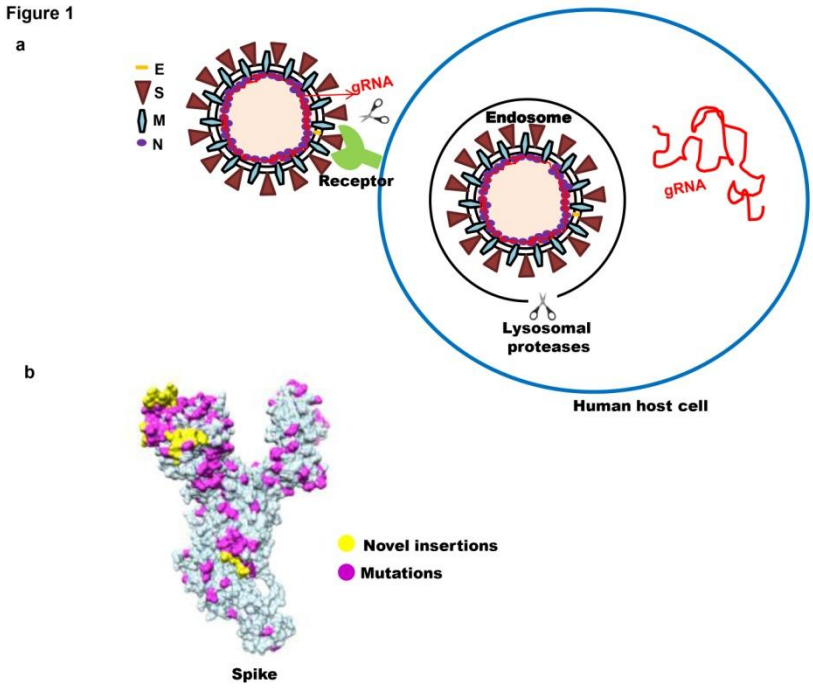

Figure 1. a) CoVs cell entry mechanisms of by endocytosis and lysosomal degradation. b) Structure of CoVs Spike. New insertions and mutations in CoV-2 Spike are highlighted in yellow and purple, respectively. Abbreviations: E= Envelope; S=Spike; M=Membrane;N=nucleocapsid;gRNA= genomic RNA. (Adapted from Shang et al., PNAS, 2020; Guzzi et al., J Clin Med, 2020).

strongly binds to its lock, the human Ace Converting Enzyme 2 (hACE2). Basically, Coronaviruses use the same entry mechanism into host cells, which begins with the attachment of the viral surface to human membrane receptors, followed by entry into endosomes and, eventually, fusion with lysosomes, which release the viral genome (figure 1a).

CoV-2 key: the Spike protein and its cleavage sites

CoV-2 Spike (hereafter CoV-2-S) is a trimeric protein, that is, *is formed by three identical units, called protomers*. It confers to the virus the "crown-like" shape, from which the term Coronavirus was coined. The structure of CoV-2-S is basically identical to that of SARS-CoV and MERS-CoV Spikes, however CoV-2-S presents a number of mutations and

Figure 2

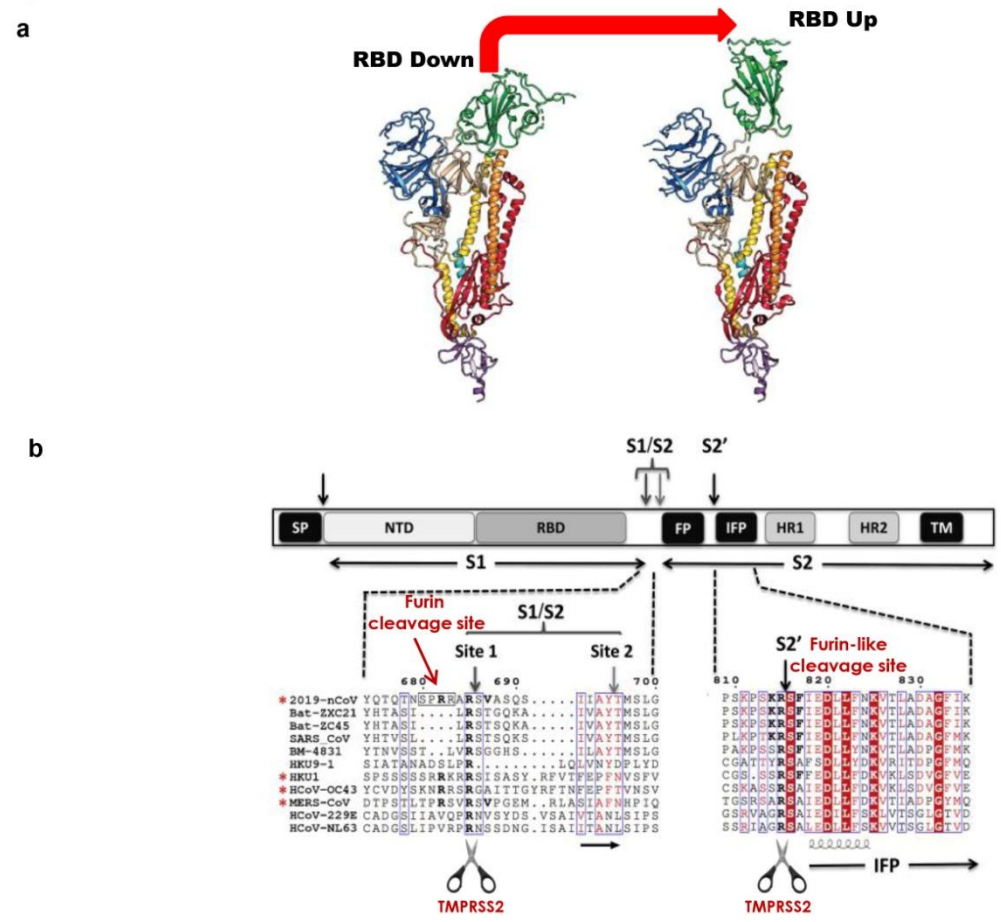

Figure 2. a) Structure of S protomer which hide or expose the RBD, in the down or up conformation, respectively. b) Schematic structure of CoV-2-S, with cleavage sites indicated by arrowheads. Downstream the scheme, an alignment with other RBD of bat and human CoVs-S is shown. Red asterisks indicate furin-like cleavage sites. Abbreviations: SP=signal peptide; NTD= N-terminal domain; RBD= receptor binding domain; FP=fusion peptide; IFP=internal fusion peptide; HR1=heptad repeat* 1; HR2=heptad repeat 2; TM=transmembrane.
**a heptad repeat is a sequence of 7 aminoacids, which in viruses is frequently found adjacent to hydrophobic fusion domains.* (Adapted from Wrapp et al., Science 2020; Coutard et al., Antiviral Res, 2020),

insertions with respect to other CoVs-S (figure 1b). CoV-2-S exists in two different conformations, called “down” or “up” (figure 2a). In the down conformation, CoV-2-S cannot mediate CoV-2 fusion with the host cell membrane. To allow virus entry, it has to undergo a conformational change, which allows the protein to acquire the up conformation[1]. This phenomenon occurs as soon as CoV-2-S approaches ACE2 through its surface domain 1 (S1), containing the receptor binding domain (RBD), after the cleavage by cellular proteases *which are proteins that cut others in the proximity of aminoacidic sequences, which are specific for each class of proteases*. This process, occurring at the S1/S2 site (figure 2b) called “priming”, generates an external domain, S1, binding ACE2, and a surface domain 2 (S2) which mediates the viral entry after a further cleavage at a site called S2’, preceding the internal fusion peptide (IFP; figure 2b). One of the cellular protease involved in CoV-2 entry into host cells is the transmembrane serine protease 2 (TMPRSS2), which has been demonstrated to be required also for SARS-CoV infection. Indeed, inhibiting TMPRS22 blocks SARS-CoV and CoV-2 entry into host cells (figure 3a)[2]. The aminoacid sequence alignment of CoV-2-S with other betaCoVs spikes shows a high grade of similarity (figure 2b). Very recently, it has been shown that CoV-2-S is 91% identical to Pangolin-CoV spike[3], indicating pangolins as the intermediate hosts of a batCoV (probably BatCoVRaTG13) and the proximal origin of human CoV-2. Nevertheless, with respect to SARS-CoV and Pangolin-CoV, CoV-2-S acquired a unique cleavage site, constituted by a stretch of basic aminoacids, *that is aminoacids with a positive charge*, targeted by a protease called furin[4,5]. This protease is termed “convertase” and its role is to activate other proteins, such as hormones and adhesion molecules[6]. A furin-like site is present also in MERS-CoV and in other human CoVs and, importantly, in highly pathogenic flu viral strains[7]. It has been recently demonstrated that, as well as TMPRSS2, furin is essential for CoV-2 entry into the host cell (figure 3b). Furthermore, also cathepsinD, which is a protease typical of lysosomes, is required for efficient CoV-2 entry. Importantly, all these protease seems to cooperate to mediate CoV-2 infection, while this is not the case for SARS-CoV, where a furin-like cleavage site is absent[8].

The human lock: ACE2 and its crystal structure in complex with CoV-2 Spike

ACE2 is one of the member of the renin-angiotensin-aldosterone system (RAAS) which maintains the homeostasis of blood pressure, electrolyte balance and inflammatory

Figure 3

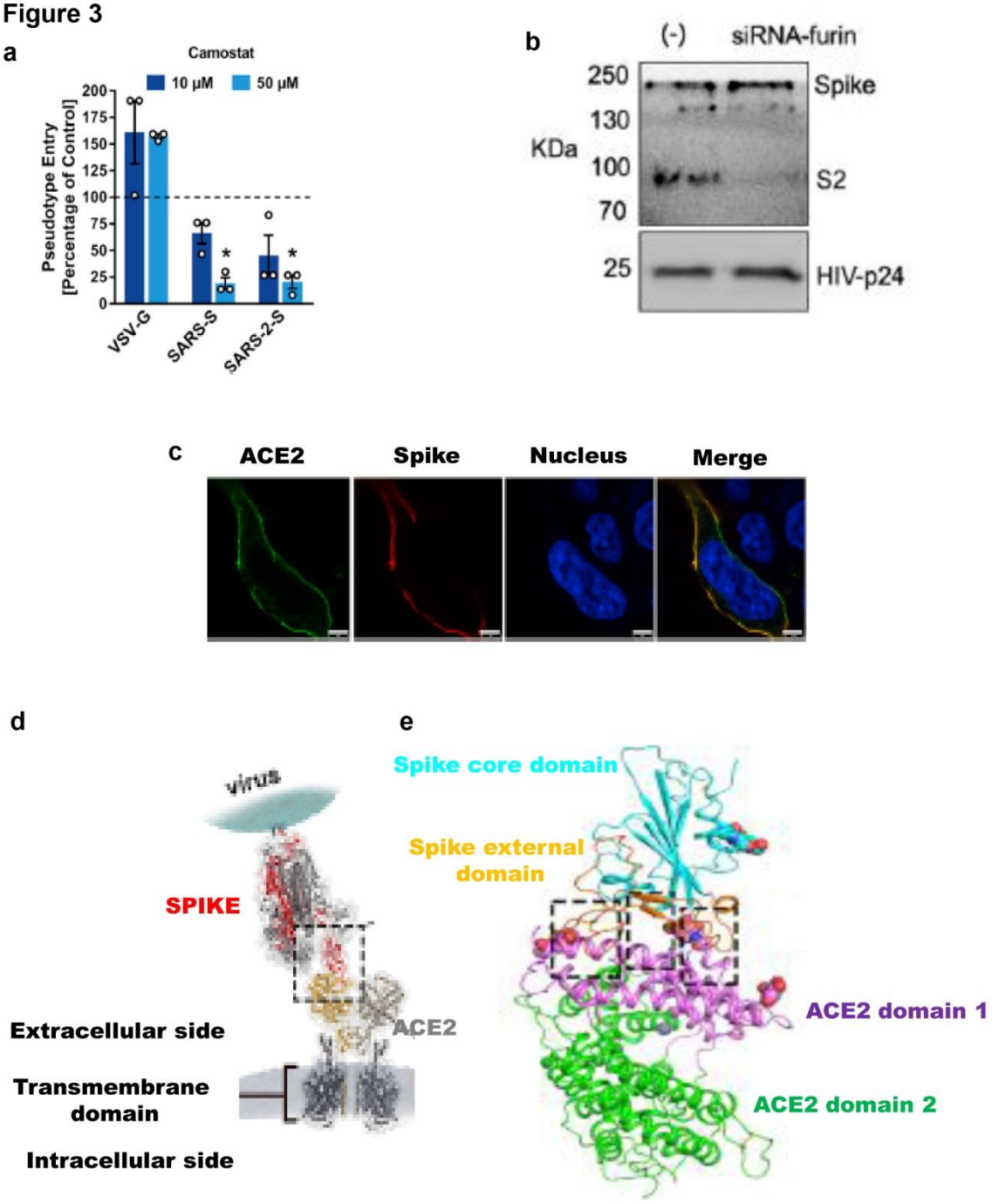

Figure 3. a) The histogram shows the impairment of SARS-CoV-S and CoV-2-S entry in the presence of a TMPRSS2 inhibitor (Camostat) at two different concentrations. b) Western blot analysis* showing that a siRNA against furin (*i.e. a molecular tool inhibiting furin production*), impairs CoV-2-S cleavage. c) Immunofluorescence** analysis showing the co-localization of ACE2 (green) and CoV-2-S (red) on host cell surface. *The overlap (merge) of ACE2 and CoV-2-S appears as a yellow line surrounding the cell membrane. Nuclei are shown in blue, by DNA labeling with a specific stain.* d) Schematic representation of hACE2 and CoV-2-S interaction on the cell surface. Interaction domains are boxed with a dash line. e) Magnification of hACE2 and CoV-2-S interactions. Specific contacts are highlighted by dash lines. Red dots represent N-glycans***. Abbreviations: VSV-G= glycoprotein of the vescicular stomatitis virus; siRNA=small interfering RNA. **Western blot is a technique which allows the visualization on a radiographic film or digital instruments of a specific protein within the cell, by the recognition of a specific antibody and the subsequent detection by a luminescent reagent. **Immunofluorescence is a technique that allows the visualization at a microscope of specific proteins within specific cell compartments, by the recognition of antibodies, labeled with different colours (in this case red and green). ***N-glycans are polysaccharides.* (Adapted from: Hoffmann et al., Cell, 2020; Wang et al., Cell, 2020).

responses, and is required for the proper function of the kidney, heart, vessels and lungs, hydrolyzing and converting angiotensin II into vasodilatatory, cardioprotective, molecules. Regarding viral-derived lung inflammation, ACE2 exerts a dual role. First, upon viral entry, ACE2 expression is reduced; second, this reduction allows soluble angiotensin II levels to increase, enhancing vascular permeability and inflammation[9]. ACE2 and CoV-2-S co-localize on the membrane of host cells (figure 3c). Recently, the crystal structure of ACE2/CoV-2-S RBD complex has been resolved (figure 3d and e), highlighting interesting differences with regards to SARS-CoV-S/ACE2 interaction. Indeed, among the 24 aminoacid of ACE2 in contact with the RBD of both CoV-S and CoV-2-S, 15 aminoacids make more contacts with CoV-2-S RBD. Furthermore, CoV-2-S interface with ACE2 presents more aminoacids that directly interact with ACE2 with respect to SARS-CoV-S interface (21 vs 17). This results in a larger surface employed in RBD/ACE2 interaction in CoV-2 with respect to that of SARS-CoV[10]. Furthermore, some aminoacid changes in CoV-2 with respect to SARS-CoV, allow CoV-2-S RBD to stronger interact with ACE2, with respect to SARS-CoV-S RBD, due to more van der waals contacts (*weak interactions among molecules which depend on fluctuations in the distribution of electric charges of aminoacids*) and salt bridges formation (*a combination of hydrogen and electrostatic bonds)*[9]. The strength of CoV-2-S-RBD interaction with ACE2 also depends on the rigidity of the interaction domains of CoV-2-S-RBD, when compared with the more flexible and unstable interaction domains of SARS-CoV RBD[11].

Putative alternate locks: integrins and CD147

Another intriguing characteristic of CoV-2-S is the presence, in the RBD but upstream to the ACE2 binding subdomain, of a RGD (R= arginine; G=glycine; D=aspartic acid) stretch (figure 4a), which is a specific recognition motif of integrins (figure 4a)[12]. Integrins are heterodimeric, ubiquitously expressed, cell surface receptors, mediating cell adhesion, migration and signaling. Interestingly, CoV-2-S RBD in the up conformation expose the RGD stretch, which is conversely hidden in the down conformation (figure 4b). This RGD motif is unique among betacoronaviruses, while other viruses, such as adenoviruses and the metapneumovirus, use integrins for cell entry. The acquisition of this motif may be evolved to provide CoV-2 an alternate mechanism of host cell entry and a larger cellular tropism. Furthermore, it has been reported that also CD147, a receptor used by SARS-CoV-S to enter host cells, may be another route of CoV-2 infection. Indeed, a humanized antibody against CD147 may prevents CoV-2 cell entry[13].

Figure 4

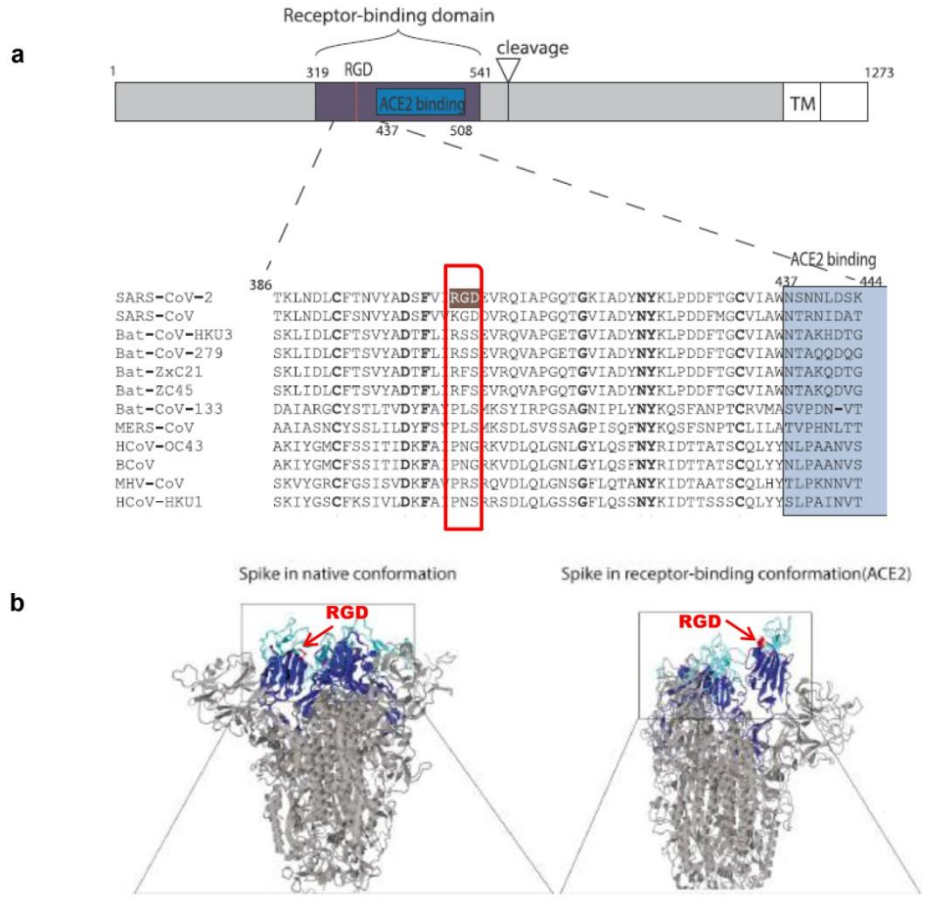

Figure 4. a) The RGD stretch is upstream CoV-2-S ACE2 binding domain and the sequence alignment of CoV-2-S with other CoVs indicate this sequence as specific for CoV-2-S. b) Structure of CoV-2-S in the down (left) or up (right) conformation: the RGD sequence (in red) is hidden when S cannot bind ACE2 and exposed when S binds ACE2. (Adapted from Sigrist et al., Antiviral Res, 2020).

hACE2 in the cardiovascular system: at the heart of a major clinical problem?

ACE2 expression in lung and bronchial cells has been well documented. Cardiovascular disease (CVD) is one of the co-morbidity conditions which makes Covid-19 prognosis unfavourable. In addition to the cytokines storm, which attacks all the organs, particularly lungs and heart, an elevated levels of TroponinT and creatin kinase (CK), which usually are detected in CVD patients, has been found to be correlated with Covid-19 severe clinical cases and high rate of mortality. A possible direct infection of cardiomyocytes by CoV-2, followed by the occurrence of myocarditis, has been postulated, but not proven by biopsies or autopsies so far[14]. However, very recently, a high expression of ACE2 has been demonstrated in the heart (figure 5a) and, specifically, in pericytes[15], accessory cells important for the proper function of the vessels and which may be a direct target of CoV-2

infection (figure 5b)[15]. This may impair the functional interaction between pericytes and endothelial cells, causing microvascular dysfunction (figure 5c), explaining the elevated

Figure 5

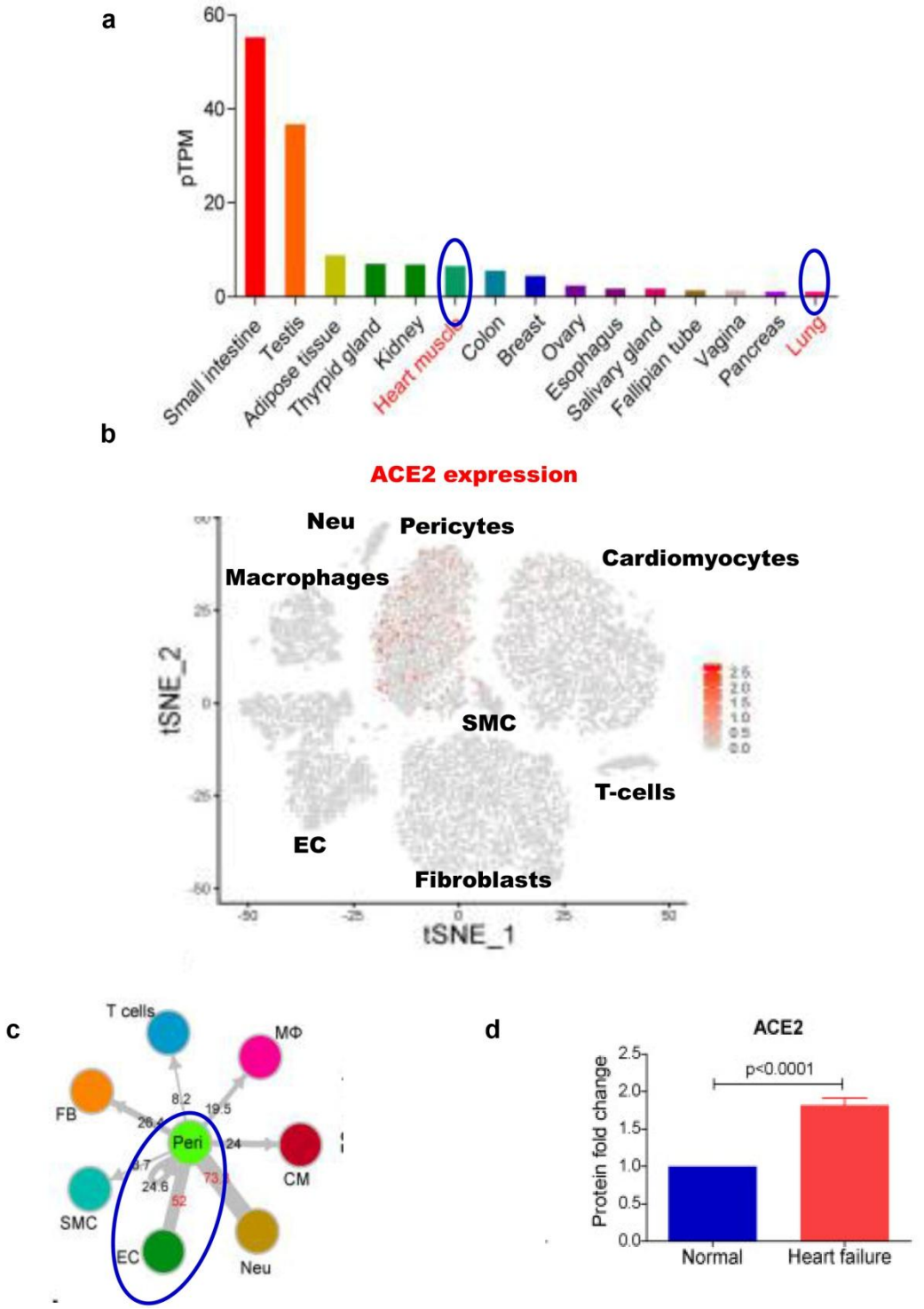

Figure 5. a) Expression of ACE2 mRNA in various tissues. Heart and lung are highlighted by blue circles. b) Representation of different cell types within the human heart and distribution of ACE2 levels (indicated by colour shading from grey to red). c) Crosstalk among pericytes and other cell types resident within the heart. The thickness of gray bars represents the strength of cell-cell interaction. Pericytes strongly interact with endothelial cells and neuron-like cells. d) ACE2 protein expression in normal and failing heart. Abbreviations: mRNA: messenger RNA; t-SNE: t-distributed stochastic neighbor embedding (t-SNE) plot, *an algorithm which picks similar objects (among a huge multitude of objects) and defines the similar probability of distribution, usually displaying clusters on a low-dimensional map. .* T cells=T lymphpcytes; MΦ=macrophages; CM=cardiomyocytes; Neu= neuronal-like cells; EC= endothelial cells; SMC=smooth muscle cells; FB=fibroblasts. (Adapted from Chen et al., Cardiovascular Res, 2020).

levels of CK, regardless the presence or absence of a cytokines storm. Further, patients with previous CVD express elevated levels of ACE2 (figure 5d)[15], which may sustain the higher susceptibility to CoV-2 infection of CVD patients, severe clinical conditions and high rate of mortality.

Conclusions

From this short review of CoV-2 entry players (Spike, ACE2, integrins, CD147, cellular proteases), mechanisms and cellular tropism, it is clear that this virus has evolved a number of additional strategy to potentiate its fitness. In particular, the possible use of alternate receptors, broaden the cellular spectrum CoV-2 may attack. ACE2 and TMPRSS2 co-localize in bronchial cells, while furin, a ubiquitous convertase, is a tool CoV-2 utilizes to more efficiently infect host cells and, together with the use of alternate entry routes, to expand host cell types. The furin cleavage site, absent in SARS-CoV, BatCoV RaTG13 and Pangolin-CoV, which are CoV-2 most proximal viruses, may have evolved in humans after the jump from pangolins, which are reported to represent the most probable missing link between bats and humans, to date. The occurrence of this polybasic cleavage site also works against the possibility that CoV-2 may be the result of a virus adaptation to repeated cell culture passages. Indeed, the acquisition of this site has been documented for three other human CoVs (HKU1, HCoV-OC43 and MERS-CoV), for high pathogenic avian influenza viruses, and for low high pathogenic avian influenza viruses, after repeated passages “in vitro” and “in vivo”. Furthermore, an ancestor virus, with high genetic similarity, should have been isolated and cultured. This viral progenitor should have acquired the polybasic site after many passages in culture, in cells expressing ACE2. This chain of events is very unlikely to be occurred. The endocytic-lysosomal path has been demonstrated to be effective also for CoV-2 entry[16] and this has clarified his precise entry mechanism. The binding of CoV-2-S RBD to ACE2 is stronger than the binding of SARS-CoV-S RBD, but, interestingly, total CoV-2-S has identical or lower affinity for ACE2 than SARS-CoV-S[8]. This may depend by the fact that SARS-CoV-S RBD, which is the most immunogenic region, is predominantly in the up conformation, while CoV-2-S RBD, is mostly in the down conformation, an observation which may also, at least partially, explain the evasion of the immune system by CoV-2. Thus, although the complex cell entry mechanism of CoV-2 appears a challenge, at the same time may suggest multiple strategies to impair cell infection.

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