Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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The Secret Life of ACE2 as a Receptor for the SARS Virus

The membrane-associated carboxypeptidase angiotensin-converting enzyme 2 (ACE2) is an essential regulator of heart function. Now, Li at al. identify and characterize an unexpected second function of ACE2 as a partner of the SARS-CoV spike glycoprotein in mediating virus entry and cell fusion.

Many cell surface-associated molecules with diverse sequences, structures, and cellular functions are usurped by viruses for use as their receptors. Receptor identification is important for understanding virus tropism, pathogenicity, and mechanisms of entry, and may help in the development of therapeutics and vaccines, but remains a challenging task. Although the number of identified receptors for human viruses has increased rapidly over the past two decades, the receptors for most of the several hundred known human viruses remain elusive. The receptor for one of the three known human coronaviruses, HCoV-229E, was identified as the human aminopeptidase N (hAPN, CD13) more than a decade ago (Yeager et al., 1992), but the functional receptor for another human coronavirus, HCoV-OC43, remains unknown. However, the overall pace of research on the third human coronavirus, the SARS-CoV, has been amazingly rapid, and, in keeping with this, just months after the virus itself was discovered, the angiotensin-converting enzyme 2 (ACE2) was identified as its receptor (Li et al., 2003).

Li et al. used a straightforward approach—coimmunoprecipitation of the virus attachment glycoprotein (S1) with lysates from cells that are susceptible to virus infection (Vero E6) followed by mass spectrometry analysis of the coimmunoprecipitated proteins. To express the SARS-CoV full-length glycoprotein (S) and S1 in sufficient amounts required for coimmunoprecipitation and functional characterization, they synthesized a codon-optimized gene based on the published sequence of the Urbani isolate (Rota et al., 2003). The observations that ACE2 specifically binds to S1, supports formation of syncytia due to cell fusion mediated by the interaction with S, and mediates infection of cells otherwise inefficient for virus replication that can be inhibited by an anti-ACE2 antibody provide convincing evidence for its receptor function.

In a remarkable series of experiments, Li et al. not only identified the virus receptor, but also demonstrated key characteristics of the membrane fusion process mediated by the ACE2 interaction with S. First, they showed that expression of recombinant ACE2 and S resulted in cell fusion at neutral pH. This finding suggests that low pH and other viral proteins are not required for fusion. The S glycoprotein from another SARS-CoV isolate (Tor2) can also mediate fusion at neutral pH (Xiao et al., 2003), suggesting that the absence of a low pH requirement to trigger fusion is not strain-specific, although more isolates should be tested. It has also been demonstrated that expression of recombinant S from some coronaviruses can lead to syncytia formation at neutral pH (Lai and Cavanagh, 1997). However, it remains possible that low pH is important for uptake of cell-free virus. Second, the S glycoprotein was not cleaved to any measurable degree, but effects of cleavage at the cell surface by proteases on fusion cannot be excluded. Recent biochemical and functional data showed that coronavirus S glycoprotein is a class I fusion protein (Bosch et al., 2003); the lack of cleavage sets apart the SARS-CoV S glycoprotein and spike proteins from other coronaviruses from a prototype class I fusion protein, which is cleaved. Third, the receptor binding domain (RBD) is within the N-terminal fragment containing amino acid (aa) residues 12–672, which Li et al. define as S1. The RBD was recently localized between residues 303 and 537 (Xiao et al., 2003) and is therefore similar to the RBD of the HCoV-229E, which is within a fragment containing residues 407 to 547 (Breslin et al., 2003); whether this reflects any similarity in structure and mechanism of binding of these human coronaviruses is unknown. Finally, Li et al. developed a fusion assay based on syncytia formation that can be used to study mechanisms and to test inhibitors without the need to work with a lethal virus. A pseudovirus-based assay would be a useful complement to control for differences between cell fusion and virus entry.

Preliminary experiments reported by Li et al. also give some initial clues to the molecular mechanism of the ACE2 interaction with S. Two mutations of the ACE2 catalytic site did not affect syncytia formation, indicating that the S binding site on ACE2 is located in a different region and that the enzymatic function of ACE2 is not required for fusion. Although normal cellular function is not usually required for a virus receptor function, further experiments are needed to validate this finding; one possible reason for the lack of effect is related to the long time (48 hr) of syncytia formation (see the supplementary information to Li et al.), which could lead to saturation. The fact that the ACE2-S1 association endured the perils of the coimmunoprecipitation procedure also suggests it may be a high-affinity interaction. The precise affinities of other coronavirus spike-receptor interactions have not been determined (Gallagher and Buchmeier, 2001). However, for most known virus-receptor interactions

(but not all), high-affinity binding suggests the possibility of receptor-induced conformational changes in the viral proteins. Whether the SARS-CoV S glycoprotein will follow this rule remains to be seen.

In trying to predict the implications of the receptor identification and future research directions, it may be useful to consider parallels with the history of HIV research. However, the speed could well be an order of magnitude faster if the research continues at the pace set by Li et al. An immediate question is whether there are other receptors or coreceptors—for HIV it took more than a decade to identify the elusive coreceptors. ACE2 is expressed at significant levels in heart and other tissues (Donoghue et al., 2000), where SARS-CoV replication has not been reported. Does it need a coreceptor(s) that is absent in cells from these tissues but not in 293T cells, or there are other factors that prevent replication in these tissues?

Perhaps the most urgent question is whether soluble ACE2 (sACE2), and various fusion constructs or fragments, can serve as potent inhibitors of the virus infection in vivo. The analogy to HIV could help to avoid costly clinical trials and save time. After the failure of recombinant soluble CD4 (sCD4) to affect HIV replication in humans, it took more than a decade to develop a potent multivalent CD4-IgG fusion protein, which is now showing promising results in recent clinical trials. Like sCD4, sACE2 is likely to have a short half-life in vivo, and may not be a very potent inhibitor in a monovalent form. Multivalent sACE2-immunoglobulin proteins might be much better inhibitors of SARS-CoV infection in vivo than sACE2. Antibodies, other proteins, and perhaps peptides and small molecules disrupting the ACE2 interaction with the S glycoprotein could also be viable tools in the treatment of SARS-CoV infections (although existing ACE inhibitors are unlikely to be useful). The solution of the crystal structure of the receptor and its complex with receptor binding fragments of S1 will provide a detailed understanding of its interactions with the viral protein and could help in the development of such inhibitors. Finally, soluble forms of the S glycoprotein ectodomain, the RBD, and even receptor-bound conformations of the S glycoprotein may have potential as vaccine immunogens that elicit neutralizing antibodies; such receptor-bound conformations of the HIV-1 gp120 have been recently proposed as vaccine immunogens that could elicit potent broadly neutralizing antibodies. The rapid pace of research and the acute self-limiting nature of the SARS-CoV infection (unlike HIV infection) could lead to significantly faster development of therapeutics and vaccines than for HIV, and this could be another unexpected but welcome surprise.

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