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The therapeutic potential of targeting ACE2 in COVID-19

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Abstract. – OBJECTIVE: ACE2 long served as the human gateway for multiple coronaviruses, including the currently pandemic SARS-CoV-2. This mini-review explores the potential of targeting ACE2 in blocking viral penetrance.

MATERIALS AND METHODS: PubMed search was conducted using the terms: "coronaviridae", "peptidyl-dipeptidase A", "ACE2", "SARS", and "SARS-CoV-2". References of relevant articles were further screened by the author.

RESULTS: Four main methods of blocking ACE2-mediated viral penetrance were identified: receptor blockage, receptor decoying, receptor shedding, and co-receptor inhibition.

CONCLUSIONS: Drugs that inhibit viral binding to ACE2 present a strong choice for the current, and if necessary, future outbreaks. Further research is needed to establish the clinical and pharmacological aspects of the identified candidate molecules.

Key Words:
Coronaviridae, ACE 2, Peptidyl-dipeptidase A, COVID-19.

Introduction

Human ACE2 (hACE2) is a single-pass-type-I membrane protein, with its enzymatically active extracellular domain acting as the binding site for the receptor binding domain (RBD) of the spike protein (SP), of both SARS-CoV and SARS-CoV-2[1]. The rationale behind targeting ACE2 is as follows:

1. Host surface proteins are less likely to mutate.
2. Viral RBDs require considerably long periods to revolutionize, making therapeutic escape less of a concern.
3. Antivirals that hinder viral penetrance present a wise choice for the current and, if necessary, future outbreaks.

Potential Methods

Blocking the ACE2

Following the 2003 SARS epidemic, scientists attempted blocking ACE2-mediated cellular infection via SARS-CoV RBD-derived peptides (Table I and Figure 1). Perhaps the most promising of these trials is the hexapeptide demonstrated by Struck et al[2], which is at least one-third the size of any of the others. A similar approach with SARS-CoV-2 RBD is also plausible, especially since it binds hACE2 much stronger[4]. This can be attributed to the fact that the novel SARS-CoV-2 utilizes an RBD within the S1 domain of its spike protein compared to an S2 RBD in SARS-CoV. Nevertheless, this comes at the expense of a lower affinity to ACE2[4].

Of potential interest, an Fc fragment attached to the recombinant RBD can prolong its half-life, as previously experimented with MERS[6], but the immune response to such a modification is unpredictable.

Decoying the ACE2

Decoy receptors are established in the management of multiple diseases. For example, the decoy of osteoprotegerin, Denosumab, is used to treat postmenopausal osteoporosis.

About a decade ago, Wysocki et al[3] developed a soluble ACE2 (sACE2) from its complimentary DNA (cDNA) encoding sequence, for purposes of cardiovascular research. Nowadays, this decoy presents a strong candidate antiviral for ACE2-binding viruses, especially due to its minimal interaction with the physiological functions of membrane-bound ACE2, in addition to its potential as a passive vaccine (Figure 1). In fact, sACE2 was recently proven, *in vitro*, to prevent the formation of the RBD-hACE2 bond in both SARS-CoV and SARS-CoV-2, binding much stronger to the latter[4].

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Table I. SARS-CoV RBD-derived hACE2 blockers.

Peptide	Sequence	Virus	References
RBD-11b	YKYRYL	SARS-CoV	3
P8	PSSKRFQPFQQFGRDVSFT	SARS-CoV	7
P9	CANLLLQYGSFCTQLNRALSGIA	SARS-CoV	7
SARSww-III	GYHLMSFPQAAPHGVVFLHVTW	SARS-CoV	8
SARSww-IV	GVFVFNGTSWFITQRNSS	SARS-CoV	8

Shedding the ACE2

Cell surface proteins undergo cycles of proteolytic ectodomain release, known as shedding, as part of their regulation. hACE2 was shown to undergo this process by ADAM17[9], a protease of the ADAM (a disintegrin and metalloproteinase) family.

In theory, not only will cleaving the ectodomain of hACE2 decrease viral binding sites, but it will also create sACE2 to bind circulating viral loads (Figure 1). However, there are multiple downsides to this approach: firstly, ADAM17 is not specific to hACE2, and was implicated in the shedding of multiple other surface proteins[10-13]; secondly, its activity is both constitutive and inducible, by phorbol esters for example, and dependant on factors like protein kinase C, intracellular Ca^{++} levels, and membrane lipid composition[13], making its pharmacokinetics rather vague; lastly, ADAM17 has been implicated in neoplastic pathophysiology[11], and efforts were made to inhibit its activity rather than induce it.

Transmembrane Protease Serine 2 (TMPRSS2) and Cathepsin L (CatL) Inhibition

Upon receptor binding, TMPRSS2 activates the S-protein of SARS-CoV to achieve cellular

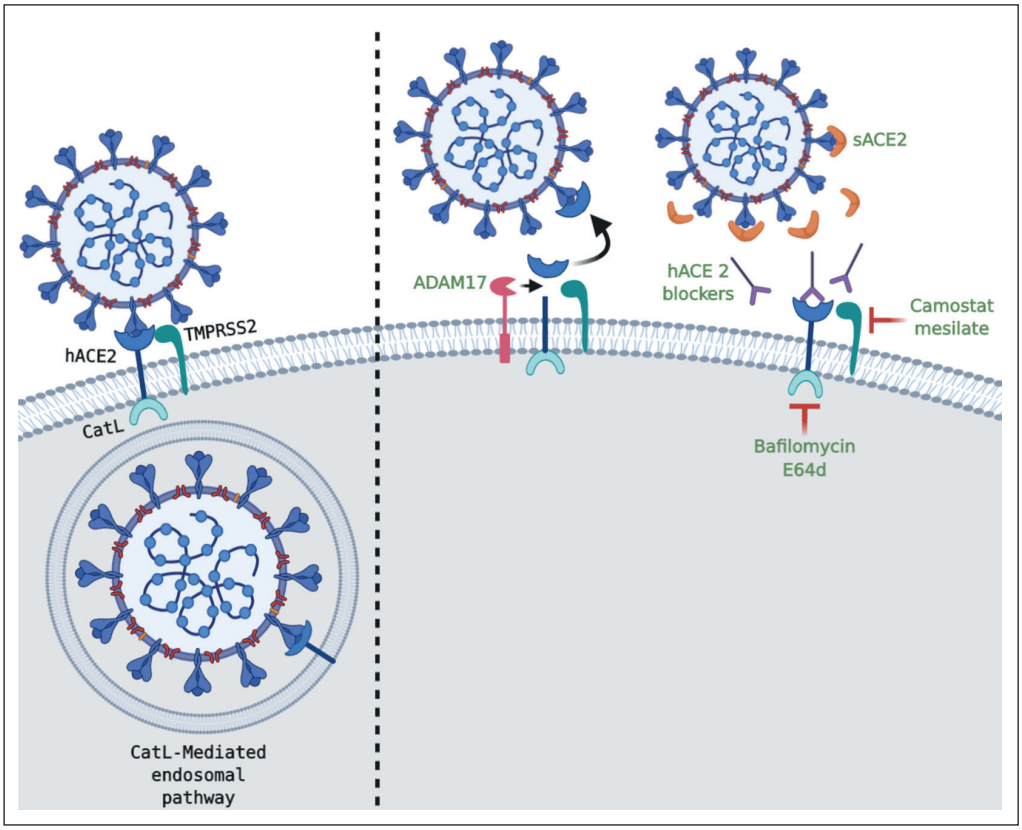

Figure 1. Interruption of viral binding to ACE2.

entry. In the absence of TPMRSS2, SARS-CoV utilizes an alternative endosomal pathway, where CatL plays the pivotal role of activating spike protein fusogenicity[14]. Hoffmann et al[15] proved that SARS-CoV-2 uses a similar mechanism. In their experiment, they achieved partial blockage of viral entry into lung cells by inhibiting TMPRSS2 alone, via camostat mesilate (CM). A complete block was only attained when E64d, a CatL blocker, was added to CM.

CM per se, is a protease inhibitor that is used to manage chronic pancreatitis in Japan, and although its mechanism is still uncertain, it proved efficacious in improving pancreatic fibrosis[16].

One can speculate that CM alone would only decrease disease severity rather than achieve complete remission in COVID-19. Thereby, the utility of CatL inhibitors should be taken into concern (Figure 1). E64d for example, is an ester prodrug which specifically inhibits cysteine proteases. The downside to it is that it rapidly hydrolyses to E64c *in vivo*, which in turn undergoes significant hepatic uptake, leaving a therapeutically unacceptable systemic dose. Nonetheless, an inhaled formulation of E64d is a potential solution for this obstacle.

Bafilomycin is another CatL Cathepsins L inhibitor that is similarly efficacious[14], it belongs to the macrolide family of antibiotics and exhibits broad biological activity including; antitumor, anti-proton pump, antiparasitic, and antifungal activity[17,18].

Conclusions

Taking into consideration the current wildlife reservoir of coronaviruses, humanity stands prone to future outbreaks by newer generations, which necessitates the search for a drug that can provide a relatively broad spectrum of action against this family.

The present article focused on methods of blocking the RBD-hACE2 bond, but further research is strongly needed to establish the clinical and pharmacological aspects of the identified molecules. Of these, the RBD-11b hexapeptide, sACE2, and CM/E64d combination seem to be the safest and most promising.

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Conflict of Interests

The author declares no conflicts of interest.

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