ACE2 fusion protein binding affinity to SARS-CoV-2 spike protein variants

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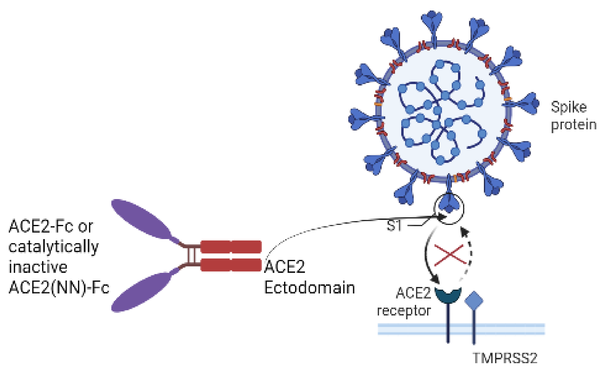
Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused the global COVID-19 pandemic. The entry of the virus into host cells is mediated by the viral spike (S) protein binding to the Angiotensin Converting Enzyme (ACE2). Currently, the FDA has approved or issued emergency use authorization for several vaccinations, small-molecule antiviral drugs, and monoclonal antibody treatments with a COVID-19 indication. However, viral variants remain a threat for the current therapeutic and vaccination efficacy. A potential therapeutic strategy to sequester these potential viral variants from host cells is to use a soluble fusion protein that contains an extracellular domain of human ACE2 fused to an immunoglobulin G crystallizable fragment (ACE2-Fc) or a catalytically inactive ACE2-Fc fusion protein (ACE2(NN)-Fc) that contains two-point mutations within the ACE2 domain. Here, we established laboratory scale processes for production and purification of ACE2-Fc and ACE2(NN)-Fc fusion proteins using Chinese Hamster Ovary (CHO) cell lines that stably express the ACE2 fusion proteins. Using these purified proteins, we characterized their identity, purity, N-glycosylation status, and binding activity to several recombinant Spike protein variants. Our results demonstrates that both ACE2-Fc and ACE2(NN)-Fc fusion proteins have binding affinity to four spike protein variants: parental, alpha, beta, delta, and omicron with different affinity. This work supports ACE2 fusion proteins as a potential COVID therapeutics that can prevent infection of SARS-CoV, including SARS-CoV-2 through blocking the binding of spike protein on viruses to its receptor ACE2.

Working Hypothesis



ACE2-Fc and ACE2(NN)-Fc fusion proteins sequester viral spike (S) protein from binding to ACE2 on host cells.

Results

Production of ACE2-Fc and ACE(NN)-Fc fusion proteins

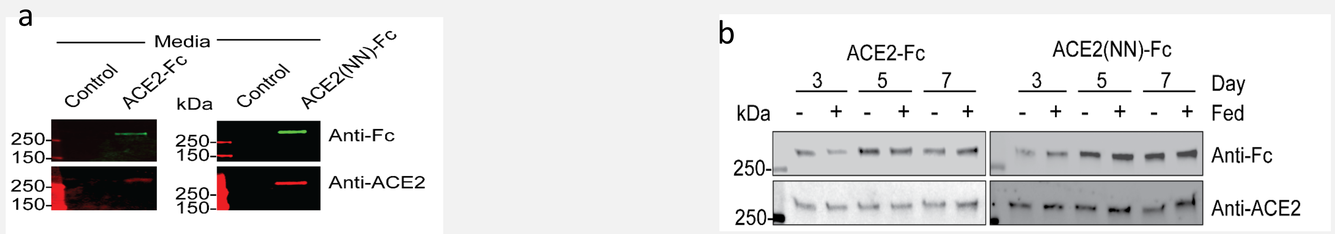


Figure 1. Analyses of ACE2-Fc and ACE2(NN)-Fc expression via IgG Fc and ACE2 immunoblotting using 15 µl of transfected CHO cell media (**a**), and protein production in a CHO cell clone over days 3, 5 and 7 days under unfed and fed conditions (**b**).

Purification of ACE2-Fc and ACE2(NN)-Fc fusion proteins

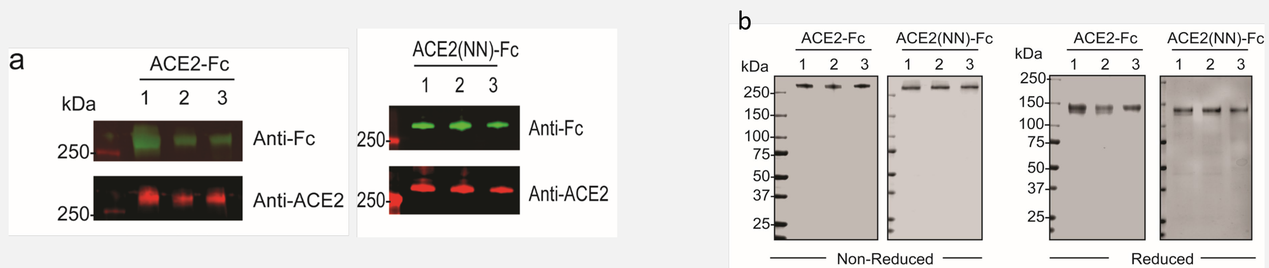


Figure 2. Identity testing of ACE2-fusion proteins using immunoblotting (**a**), and purity testing via non-reducing and reducing SDS-PAGE with Coomassie staining (**b**).

N-glycan Structures of ACE2-Fc and ACE2(NN)-Fc fusion proteins

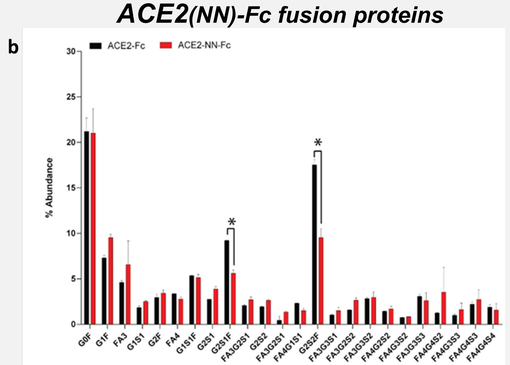


Figure 3. N-Glycan Analysis: Relative abundance of different N-glycans of each N-glycan from ACE2-Fc and ACE2(NN)-Fc proteins.

Binding affinity of ACE2-Fc and ACE2(NN)-Fc fusion proteins to spike protein variants

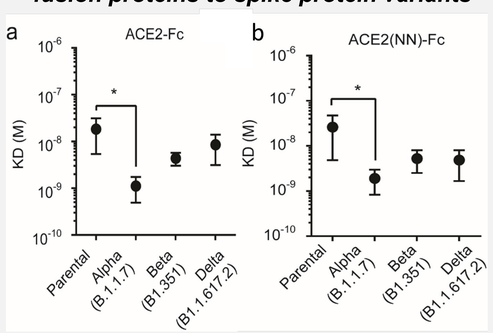


Figure 4. The binding affinity of ACE2-Fc (**a**) and ACE2(NN)-Fc (**b**) fusion proteins to four different spike protein variants measured through BLI analyses.

Conclusion

ACE2-Fc and ACE2(NN)-Fc proteins bind to spike protein variants.

Disclosures

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