Identification of Tripeptide Modulators of ACE2 Activity Using a High Throughput Screen

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Introduction

Angiotensin converting enzyme 2 (ACE2) works in the renin angiotensin aldosterone system to decrease circulating levels of angiotensin II by removing the C-terminal phenylalanine and converting it to angiotensin (1-7).[1] In addition, ACE2 has received increased interest in research due to its role in COVID-19 pathogenesis, as the binding site and cell entry gate for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[2] While ACE2 inhibitors have been primarily used as pharmacological tools to study the renin-angiotensin system, small molecule ACE2 enhancers (aka activators) are highly desired because of their hypothesized therapeutic potential.

Many attempts have been made to identify an ACE2 enhancer for this reason.[3] Currently there has been no reproducible activator reported in literature.[1,4] This study was designed to identify peptide-based enhancers of ACE2. The goal of testing all possible tripeptide combinations of the 20 proteogenic amino acids as a high throughput screen was to expedite the time requirements of individual testing. It was believed that this screen could be an inroad to guide further refinement of activators through biopharmaceutical models in an efficient and cost-effective manner

Aims and Objectives

AIM I: To determine the effect of a library of polypeptides on the activity of recombinant ACE2 using enzymatic assays

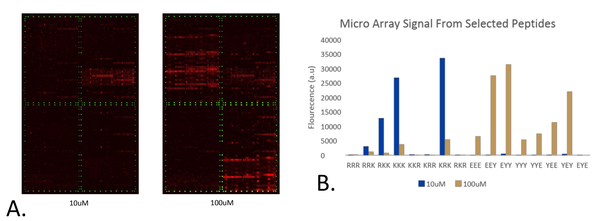
AIM II: To document the interaction of select polypeptides with recombinant ACE2 using biophysical and biochemical assays

Methods

First, binding of human recombinant ACE2 to all possible tripeptides composed of the 20 proteinogenic amino acids, was evaluated using a proprietary immunofluorescence-based peptide microarray. Binding of 6xHis-tagged ACE2 to the 8000 tripeptides immobilized on a microchip was evaluated at 10 µg/ml and 100 µg/ml concentrations of the peptidase using a DyLight680-conjugated anti-6xHis-tag antibody. Hemagglutinin (HA) immobilized on the microchip served as a positive control peptide in the microarray and it was tracked using a DyLight800-conjugated anti-HA antibody. The read-out was performed with an Innopsys InnoScan 710-IR Microarray Scanner at scanning gains of 50/10 (red/green). In the result of the microarray a number of tripeptides were identified as potential ACE2 binders.

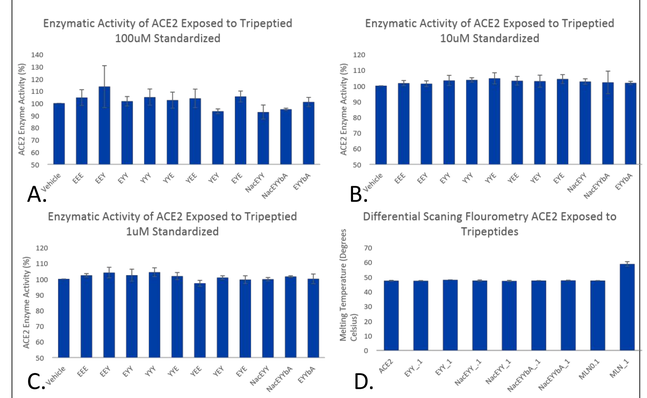
Among them, 22 tripeptides were selected that resulted in strong signal as well as similarly structured peptides with low signal to act as control. The effect of the selected peptides (at 1, 10 and 100 µM) on activity of human recombinant ACE2 was tested in a continuous enzymatic assay using a fluorogenic substrate. Differential scanning fluorometry (DSF) was used to determine whether these peptides physically interact with recombinant ACE2 with MLN-4760 used as a positive control. Data analysis and collection were conducted in a double blinded fashion.

Results

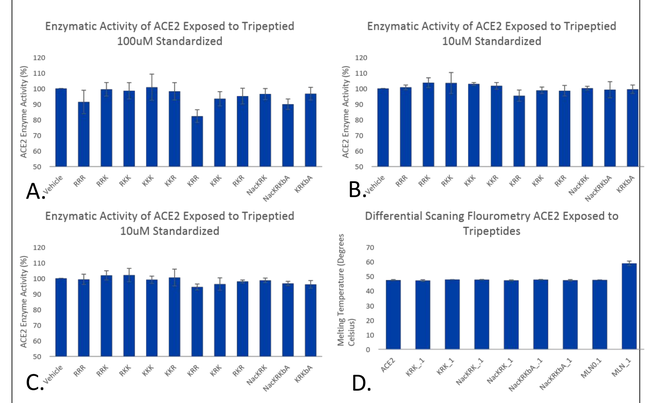


A. Elisa MicroArray of 8000 tripeptide combinations of 20 proteinogenic peptides with the background corrected. Geen signal is originating from HA protein control. Red signal is originating from anti-6xHis-tag antibody. Results from microarray with 10uM concentrations and 100uM concentrations of tripeptides.
B. Chosen peptide sequences for further testing with their corresponding signal after correction for background and variance. Peptides are noted in their single letter abbreviations.

Results



A.,B.,C., Enzymatic activity ACE2 displayed as the slope of real time fluorescence assay normalized to the slope of ACE2 exposed to vehicle expressed as a percentage. ACE2 exposed to 100uM, 10uM, 1uM tripeptide solution. Tripeptides tested are noted using their single letter abbreviations. Pvalues >0.05. Standard deviation displayed.
D. Differential scanning fluorimetry of ACE 2 exposed to vehicle, tripeptides, and MLN-4760. Peptide concentrations are displayed as .1 for 10uM, and 1 for 100uM. Mean peak intensity for ACE 2 was 47.25°C (SD, 0.29), mean peak intensity of MNL-4760 was 47.50°C (SD, 0) at 1uM, and 58.83 °C (SD, 1.59). Pvalues >0.05.



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D. Differential scanning fluorimetry of ACE 2 exposed to vehicle, tripeptides, and MLN-4760. Peptide concentrations are displayed as .1 for 10uM, and 1 for 100uM. Mean peak intensity for ACE 2 was 47.25°C (SD, 0.3), mean peak intensity of MNL-4760 was 47.50°C (SD, 0) at 1uM, and 58.83 °C (SD, 1.6). Pvalues >0.05.

Conclusions

The microarray chip appeared to show show characteristic clustering around lysine rich peptide with a preference for KxK sequences at 10uM. Such interaction can be driven ionic interactions. The assay again showed grouping at 100uM for acidic and aromatic peptides E and Y. This interaction could be driven by ionic interactions and π-stacking.[5] No repeat testing was preformed of the ELISA microarray, and further testing can be done to characterize the displayed interaction.

Through the high throughput screen KKK, KRK, EEY, EYY, and YEY were identified for their high reported signal. Also from the original high throughput screen RRR, KKR, KRR, RKR, and EYE were chosen due to their low reported signal and were expected to act as negative controls. Remaining peptides RRK, RKK, EEE, YYY, YYE, YEE were selected for their structural relation to the highest signal tripeptide sequences. The groupings of RRK, RKK, KKK, then, EEE, EEY, EYY, as well as YYY, YYE, YEE, and YEY appeared to have sequence dependent changes in fluorescent intensity. KRK, and EYY showing the highest signal were also synthesized with N-acytelated beta alanine versions to examine if the linker sequence for the microarray plate was the cause for reported signal and hypothesized ACE2 interaction.

The results of the enzymatic activity assay suggested no statistically significant interaction between the 22 tested peptides and ACE2 in a way that interfered with ACE2 activity. However, interaction between ACE2 and the tripeptides could still occur that does not affect enzyme activity which would explain the ELISA results. DSF was used to assess changes in enzyme denaturation and showed no statistically significant change in 6 of the 22 peptides.

In conclusion the high throughput microarray test of ACE2 under further examination did not result in a peptide sequence that showed interaction with ACE2 structure or function. The discrepancy of testing could be due to the methods used and confounding variables listed above. Further research into the field of ACE2 activators should be conducted due to it's promising pharmaceutical potential. Other untested tripeptide sequences are available from the assay and present an avenue for further research.

References

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