COMBATTING FUTURE BIOLOGICAL THREATS – HOST-DIRECTED INTERVENTIONS TO EMERGING THREATS FOR RAPID RESPONSE

Identification Of Countermeasures Against Viral Replication By Targeting Viral Entry-related Host Proteins

Joshua Breidenbach Biochemistry and Biotechnology Group, Bioscience Division, Los Alamos National Laboratory
Shepard Moore Physical Chemistry and Applied Spectroscopy Group, Chemistry Division, Los Alamos National Laboratory
Alba Montoya Biochemistry and Biotechnology Group, Bioscience Division, Los Alamos National Laboratory
Brian Jun Biochemistry and Biotechnology Group, Bioscience Division, Los Alamos National Laboratory
Jennifer Harris Physical Chemistry and Applied Spectroscopy Group, Chemistry Division, Los Alamos National Laboratory
Tyler Reddy Department of Theoretical Biology and Biophysics Group, Los Alamos National Laboratory
Karissa Sanbonmatsu Department of Theoretical Biology and Biophysics Group, Los Alamos National Laboratory

Inadequate predictive tools coupled with high-throughput screening workflows hindered the rapid development of countermeasures to mitigate the devastation caused by the COVID-19 pandemic. With increasing globalization and population density, another pandemic becomes more likely. Any viral life cycle requires: viral attachment, internalization into the host cell, biosynthesis of viral components, maturation/assembly, and finally lysis. Therefore, preventing attachment and viral host cell entry can halt the infection cycle. Viral particles utilize host cell membrane molecules for binding specificity, and the interactions with the host membrane components trigger activation of the particle and fusion with the host membrane. Fortunately, these host membrane components are usually highly conserved in contrast to the rapidly evolving virus and thus present well characterized targets for intervention.
Utilizing SARS-CoV-2 and the broad coronavirus family as an example, we took advantage of the viral targeting system by synthesizing peptides which mimic the substrates of host cell membrane protein interactions. These peptides are meant to disrupt viral attachment and fusion until they are naturally cleaved by the host cell, slowing viral replication without irreversibly inhibiting the host cell functions. We aimed to develop high-throughput cell culture-based screening workflows to test the safety and efficacy of our potentially inhibitory peptides. First, we performed an analysis of published gene expression to compare the presence of viral entry-related host proteins in traditional cell culture models of airway epithelium versus the contemporary 3D air-liquid interface (ALI) methods. A fusion assay was established, in which human cells mimic the isolated activity of viral attachment and fusion. In addition, fluorescent staining was utilized to monitor cytotoxicity. Peptides were tested over a broad range of concentrations (10-3 to 103 μM) and fusion inhibition and cytotoxicity were evaluated by microscopy in real-time followed by image analysis methods.
For SARS-CoV-2, only slight differences were found between the viral entry-related host protein expression of traditionally cultured cells versus the 3D ALI cell cultures. Importantly, our methodology revealed several peptides with fusion inhibition activity but without detectable host cytotoxicity. Follow-up testing will be performed to determine if these peptides will have efficacy in slowing the replication of multiple viral strains and species within the coronavirus family.
Taken together, these experiments revealed that treatment with peptides mimicking the host membrane activities is a plausible method for inhibiting viral entry and likely slowing the progression of disease and providing essential time for the host immune system to clear the infection. These peptides are thus readily available first line countermeasures and can serve as lead compounds for the development of host-based antivirals. In a broader sense, we believe this strategy and the workflows established can be essential for the fight against the next viral pandemic.

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