Examining the SARS-CoV-2, 3CLpro, clinical mutants for drug resistance

Authors: Sydney Beechboard[1], Emma K. Lendy[1], Uttara Jayashankar[2], Arun K. Ghosh[3], Andrew D. Mesecar[1]

[1]Department of Biochemistry, Purdue University, West Lafayette, IN, USA

[2]Department of Biological Sciences, Purdue University, West Lafayette, IN, USA

[3]Department of Chemistry, Purdue University, West Lafayette, IN, USA

Abstract: *This project examines the kinetic and structural characteristics of 3CLpro clinical variants to investigate potential drug resistance of the SARS-CoV-2 virus drug.*

In 2019, the emergence of SARS-CoV-2 infecting the human population caused a global pandemic. According to the World Health Organization, the current death toll for the disease cause by this virus, COVID-19, has reached nearly 6.9 million deaths. While early efforts to develop treatments led to available vaccines and antiviral drugs, SARS-CoV-2 still kills hundreds of people daily. With the continued circulation of this virus and the use of Paxlovid, an antiviral inhibitor of the 3-Chymotrypsin-like protease (3CLpro), it is expected that SARS-CoV-2 variants containing drug resistant mutations within 3CLpro will develop. This study examines the catalytic properties of different SARS-CoV-2 3CLpro mutants and the ability of these mutants to be inhibited by several different inhibitor scaffolds.

The Coronavirus3D database was utilized to identify 3CLpro clinical mutations. Based on their location within the active site and their high prevalence within the human population, five mutants were evaluated in this study: M49I, M165I, Q189K, P168S, and N142S. After expression and purification, the mutants' kinetic activities were assessed using a Förester Resonance Energy Transfer (FRET)-based cleavage assay. The ability of these mutant proteases to recognize and cleave the peptide substrate was determined by measuring their catalytic efficiencies via their apparent (k_{cat}/K_m) values. Surprisingly, the catalytic efficiencies of 4 out of 5 mutants examined were lower than the wild-type protease, with Q189K exhibiting a 10-fold reduction in catalytic efficiency. The same assay also evaluated the potencies of several compounds that show low nanomolar inhibition of 3CLpro WT. The mutant M165I had a higher K_i than WT for many inhibitor scaffolds and a 44-fold increase for the inhibitor, GRL-051-22. This work lays the foundation for kinetic evaluation of clinically relevant SARS-CoV-2 3CLpro mutations to assess their effect on inhibitor efficacy and create more personalized treatment plans for individuals diagnosed with COVID-19.