Structural Studies of Non-structural protein 15 (Nsp15) Endoribonuclease from Original SARS-CoV-2 and its Variant Epsilon for Therapeutic Intervention

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SARS-CoV-2 is responsible for the ongoing global pandemic of COVID-19. Since first identified in Wuhan, China in December 2019, SARS-CoV-2 has caused 6.6 million deaths worldwide. The ongoing COVID-19 pandemic has shown the urgency to investigate the structural and functional aspects of important viral proteins for the development of more effective vaccines and therapeutic drugs. Nsp15, an endoribonuclease from SARS-CoV-2 plays active roles in immune evasion and hence emerged as a drug target for COVID-19. Here we report the identification and characterization of a high-frequency mutation in Nsp15 from the SARS-CoV-2 variant, Epsilon. First detected in California, USA in July 2020, Epsilon exhibited increased transmissibility compared to other SARS-CoV-2 variants circulating at the time. We performed multiple sequence alignments of 126 genomes of epsilon and identified four non-synonymous amino acid changes in Nsp15 (V66L, A81V, D183N, and E266Q). We created these four single mutants to study their impact on Nsp15 catalysis. We also created a catalytically inactive mutant (H234A) for comparative analysis. Initial protein expressions revealed E266Q which had a mutation rate of 4.76% exhibited very low expression levels compared to wild type (WT) whereas the inactive H234A exhibited very higher expression levels. This observation was reiterated by large-scale protein purification as well. We hypothesize that being an endoribonuclease, Nsp15 might be cleaving its own mRNA during recombinant expression, thus the activity levels of E266Q, WT, and H234A directly correlate to their corresponding expression levels. We performed a preliminary activity assay which demonstrated catalytic efficiency of E266Q is equal to or even slightly higher than WT. Enzyme kinetics experiments are underway to confirm this phenomenon. Currently, we are pursuing comparative structural studies on E266Q, WT, and H234A using both cryo-electron microscopy (cryo-EM) and X-ray crystallography techniques to understand how a mutation in Nsp15 influences increased transmissibility of SARS-CoV-2. This will lead to mitigation strategies and therapeutic interventions not only against Epsilon but also against other emerging variants.