Review: Receptor binding, immune escape, and protein stability direct the natural selection of SARS-CoV-2 variants (Upadhyay *et al*, 2021)

by Fiona Walter

The paper "Receptor binding, immune escape, and protein stability direct the natural selection of SARS-CoV-2 variants" by Upadhyay *et al.* (2021) experimentally investigates some common drivers of natural selection to determine which of these drivers guide natural selection in SARS-CoV-2[1], the RNA virus causing COVID-19[2]. The authors outline that, whilst SARS-CoV-2 mutates less frequently than other RNA viruses[3], it poses an increasing risk to humans as the current mutations are relating to faster infectivity amongst humans[4]. Particularly mutations in the receptor binding domain (RBD) of the spike protein are attributed to this increased infectivity[4] due to the RBD's role in binding to ACE2, an enzyme present on host epithelial cells[5]. The mRNA of the RBD or the whole spike protein are mostly used to make COVID-19 vaccines[6]. Therefore, this paper aims to understand how mutations in the RBD shape the viruses' evolutionary path to inform the further development of therapeutic methods to control this dangerous pathogen.

The authors examined how variants with RBD-mutations affect RBD-protein expression, RBD stability, the variants' affinity to ACE2 and its ability to escape from host antibodies. To generate these variants, the amino acid sequence of the SARS-CoV-2 RBD was downloaded and synthesized by a biosciences company. The 12 most common variants were determined using computational analyses and, to reflect these variants, the received RBD sequences mutated using site-directed mutagenesis. Amongst these were single, double- and triple-mutants. The synthetic variants were cloned into a constitutive mammalian expression vector with a His-Tag to allow for RBD-protein expression and stability analyses. The ACE2-protein was also obtained from the same company and, upon receival, transfected into human cells to enable testing of RBD-ACE2 affinity and RBD antibody-escape.

To test for RBD protein expression of the variants, the authors expressed the protein in human embryonic kidney (HEK) cells and ran the extracted protein on SDS-PAGE. This showed that all but one mutant had protein expression differing from the wild-type. Most notably were the triple-mutant which showed a much higher protein expression than the wild-type. The experiments were continued with 8 of the 12 mutants as four of the mutants showed no protein expression at all. One of these was a triple-mutant, and the rest were single-mutants. Using far-UV circular dichroism (CD) spectroscopy, the global protein structure was assessed. Here, all mutants had a similar structure to the wild-type. However, the authors suspected there may be differences in protein thermal stability, despite the similar protein structures. Therefore the authors denatured and re-folded the proteins using thermal exposure whilst monitoring the structure with the far-UV CD spectroscopy method. This showed that the thermal stability of all mutants was also similar to that of the wild-type.

Finally, the authors evaluated RBD mutants' binding affinity to ACE2 and their ability to escape from host antibodies. Here, the interaction between RBD and ACE2 was assessed using isothermal titration calorimetry (ITC). The results illustrated that all but two mutant's affinity to ACE2 was increased. The highest affinity was observed in one single-mutant as well as the triple-mutant which had the same mutation as this single-mutant. To determine RBD mutant's antibody escape, the authors exposed the RBD mutants to antibody CC12.1, an antibody isolated from a COVID-19 survivor[7], and measured RBD-CC12.1 binding using ITC. Three of the single-mutants and the triple-mutant had increased ability to escape from the hosts' antibody.

In this paper, the authors claim that the results illustrate that RBD mutations in protein stability, receptor binding and immune escape partially direct the natural selection of SARS-CoV-2, and therefore its evolutionary path. They argue that the virus has found a stable protein structure (at least for the spike protein) and natural selection is driven by mutants that maintain this structure whilst having increased affinity to host tissue or/and an advanced immune evasion. Thus, indicating that natural selection is guided by several factors rather than one key factor. A notable issue in this study is that the authors are assessing common drivers of natural selection, yet they are not comparing these to a selectively neutral factor. This means that, whilst all the observations about current SARS-CoV-2 variants may be accurate, it is not possible to verify within this study that the factors are objectively favourable for natural selection, or whether any other factor could show the same results.

Considering this significant issue, the claims of the study are perhaps not accurate. However, I believe the results still have an important message to convey. Over the past 2 years that SARS-CoV-2 has emerged and evolved[2], the vaccines' effectiveness has decreased[8]. Most known vaccines are based on the mRNA of the RBD or the whole spike protein[6], and this study demonstrates that mutations within these specific elements affect natural selection. It illustrates that, despite SARS-CoV-2 being a naturally slower evolving virus, it is still a virus capable of "adapting to its circumstances". Therefore, this study provides us with an explanation of why the vaccines are becoming increasingly less effective. Even though it was only generally suggested by the authors that this paper informs therapeutic development, I believe it is important to specifically name one key message: this study showed that it may be necessary to adapt vaccines based on variants that are predicted to emerge, similar to the annual adaptation of the Influenza-vaccine[9].

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