Rapid Reviews COVID-19

Review 2: "Protein arginylation is regulated during SARS-CoV-2 infection"

Aaron Smith¹

**¹Assistant Professor University of Maryland Baltimore County Chemistry and Biochemistry
UNITED STATES "**

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RR:C19 Evidence Scale rating by reviewer:

- **Potentially informative.** The main claims made are not strongly justified by the methods and data, but may yield some insight. The results and conclusions of the study may resemble those from the hypothetical ideal study, but there is substantial room for doubt. Decision-makers should consider this evidence only with a thorough understanding of its weaknesses, alongside other evidence and theory. Decision-makers should not consider this actionable, unless the weaknesses are clearly understood and there is other theory and evidence to further support it.

Review:

Arginylation is an important but underappreciated post-translational modification (PTM). Catalyzed by the eukaryotic enzyme ATE1, the PTM arginylation is beginning to emerge as an essential regulator of intracellular homeostasis—not only through its degradative properties in tagging proteins for ubiquitination and proteasomal degradation via the N-degron pathway— but also through its non-degradative control of protein stability and oligomerization. Coming fresh off the heels of recent revelations that arginylation is important in maintaining the stability of *Lentiviruses*, such as the human immunodeficiency virus (HIV), it is perhaps unsurprising to find that the level of arginylation may be regulated by infection of *Coronaviruses* like SARS-CoV-2.

This preprint by Macedo-da-Silva et. al demonstrates the effects of SARS-CoV-2 infection on eukaryotic intracellular arginylation. Using several model cell lines, such as kidney epithelial cells (Vero E6) and lung adenocarcinoma (Calu-3 cells), cellular protein arginylation was studied over two days post-SARS-CoV-2 infection. Basal levels of enzymes involved in the N-degron pathway and their levels post-infection were assessed in human and monkey cell lines. Unfortunately, despite the large size and amount of the data collected, very little data are actually presented in the preprint; rather, some broad conclusions are presented (e.g., Figs. 2, 3, 5). In general, it appears that infection of SARS-CoV-2: a) does increase ATE1 expression (except in macrophages); b) displays a corollary between increases in ATE1 expression and several endoplasmic-reticulum centered events; c) shows a change in arginylation-related proteins associated with the cytoskeleton, cytoplasm, and nucleus; and d) does not increase ATE1 in patient-derived macrophages but does increase *BiP* and *hspA5*

gene expression. These effects seem to be limited to the *Coronaviridae* family; however, actual data to support this statement appear to be lacking in Fig. S5. Indeed, the lack of available data effectively requires the reader to put trust in the interpretation of the authors where they should otherwise convince the reader that these statements are true.

One large weakness of the work lies in the exploration of the inhibitors of ATE1-catalyzed arginylation such as tannic acid and merbromin. While the authors show limited data that the viral load in Calu-3 cells treated with tannic acid and merbromin is diminished, it is unclear (and not presented) as to whether these inhibitors actually prevent virus entry into the cell or instead affect further downstream processes. Unfortunately, the current understanding of how tannic acid and/or merbromin inhibit ATE1 is completely unknown at the atomic level. It is also unclear whether tannic acid is capable of entering the cell and having a direct or (rather) indirect effect on ATE1 function. Moreover, while merbromin is a known antiseptic, the presence of the mercury moiety may have off-target effects on other Cys-rich intracellular proteins. The authors claim that the cellular viability was not affected by either inhibitor (although the data are not shown), but what may occur after long-term exposure to either of these inhibitors is unknown and untested. Thus, readers should be extremely cautious as these compounds are neither approved nor explored clinically as SARS-CoV-2 infection inhibitors, and thus more work is absolutely necessary to validate this aspect of the preprint.