Review 1: "The glycosylated extracellular domain of MUC1 protects against SARS-CoV-2 infection at the respiratory surface"

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RR:C19 Evidence Scale rating by reviewer:

- **Reliable.** The main study claims are generally justified by its methods and data. The results and conclusions are likely to be similar to the hypothetical ideal study. There are some minor caveats or limitations, but they would/do not change the major claims of the study. The study provides sufficient strength of evidence on its own that its main claims should be considered actionable, with some room for future revision.

Review:

The work presented by Chatterjee and colleagues investigated the role of the transmembrane MUC1 mucin in pulmonary epithelial cells upon SARS-CoV-2 infection. The authors show that removal of the extracellular domain of MUC1 by the O-glycan specific mucinase, StcE, greatly enhances viral entry—further underlining an essential role for MUC1 in the host defence that restricts SARS-CoV-2 infection. The methods and statistical analyses used are appropriate to support the main findings and the conclusions of the manuscript are interesting and well-formed. However, there are some concerns regarding this study that should be further addressed in the discussion section.

First, this study highlights the critical importance of MUC1 in limiting SARS-CoV-2 infection by steric hindrance and points towards a strategy to promote MUC1 expression—thereby boosting mucosal host defence mechanisms. However, other studies focus on the reduction of mucin expression since mucin hypersecretion is seen in critically ill COVID-19 patients with MUC1 overexpression as a major discriminant for a severe course of the disease. Furthermore, SARS-CoV-2 infection triggers mucin expression in pulmonary epithelial cells. Thus, one would expect that this virus utilizes mucins to facilitate its infection. Is there evidence that SARS-CoV-2 binds to mucins or induces a mechanism by which the highly glycosylated extracellular domain of MUC1 is removed upon infection to ease its adherence to the ACE2 receptor? Besides restricting microbial invasion at the surface epithelium, transmembrane mucins are also involved in intracellular signal transduction. Is there a difference in ACE2 expression in the presence or absence of the MUC1 extracellular domain?

Second, the authors mainly focus on MUC1 without investigating other mucins, such as MUC13 and MUC21, which are also expressed by Calu3 cells. Although both

MUC13 and MUC21 have much shorter extracellular domains, would it be expected that these mucins also serve as SARS-CoV-2 restriction factors in Calu3 cells?

Finally, ACE2-expressing Calu3 cells are frequently used to investigate viral infection. As Calu3 cells are lung adenocarcinoma cells, these results can also be of interest for lung cancer patients susceptible to COVID-19. Verifying the results of this study in normal human bronchial epithelial cells would be of added value for non-cancer patients.

I therefore suggest that the authors elaborate more on their findings with existing literature and highlight the abovementioned limitations of their study.