Evidence of increased Cathepsin B/L and decreased TMPRSS2 usage for cell entry by the SARS-CoV-2 Omicron variant

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Letter details:

Main Text: ~1200 words; Figures: 1; References: 10

The SARS-CoV-2 Omicron variant appears to exhibit altered cell tropism and entry properties compared to other variants. Here, analysing recent data, we found a strong positive correlation between the entry efficiency of the Omicron variant and the relative usage of the host protease Cathepsin B/L by the original SARS-CoV-2 strain for cell entry. We developed a mathematical model to quantify entry efficiency in *in vitro* assays and found that the Omicron variant displayed >4-fold improved efficiency in using Cathepsin B/L for entry in 293T-ACE2 cells compared to the original strain. The preferential usage of Cathepsin B/L over TMPRSS2 for entry may explain the altered cell tropism of the Omicron variant and have implications for our understanding of its infectivity and transmissibility as well as for interventions.

The new SARS-CoV-2 variant Omicron (B.1.1.529), declared a variant of concern on 25th November 2021, is fast becoming the dominant strain in several countries. It harbours as many as 37 mutations in the spike protein compared to the original SARS-CoV-2 strain [1] and is able to evade neutralising antibodies generated by previously infected or vaccinated individuals [1-3], possibly leading to its increased transmissibility and rapid global spread. Emerging data indicates that the high number of mutations in the Omicron variant affects its viral entry properties and cell tropism [2-5], which in turn may influence its ability to establish infection post-exposure and the severity of the subsequent symptoms. In this letter, we analyse this new data and find that the Omicron variant has substantially altered preferences for cell entry pathways compared to the earlier variants. Further, using a mathematical model, we quantify the extent of the differential usage of the entry pathways by the Omicron variant.

Early studies showed that the original SARS-CoV-2 (Wuhan-Hu-1) strain displayed broad cell tropism, with viral entry a key determinant of the tropism both *in vitro* [6] and *in vivo* [7]. Intriguingly, multiple recent *in vitro* studies suggest that the Omicron variant's cell

44 tropism may be altered and its entry efficiency different from the original and other variants in
45 a cell line-dependent manner [2-5]. For instance, SARS-CoV-2 pseudotyped virus bearing
46 either the B.1 or the Delta variant spike protein showed higher entry efficiency than the
47 Omicron pseudotyped virus in Caco-2 (human, colon) and Calu-3 (human, lung) cells, whereas
48 the Omicron pseudotyped virus entered Vero (African green monkey, kidney) and 293T
49 (human, kidney) cells more efficiently than the other variants [3]. Similar trends were observed
50 in live SARS-CoV-2 virus infection assays [4, 5]; the Delta variant infection spread was
51 significantly greater than the Omicron variant in Calu-3 cells [4, 5], whereas the spread of the
52 two variants was similar in VeroE6 cells [4]. What causes the relative efficiency of Omicron
53 virus entry to be higher in some cells and lower in others than other variants?

54 The first step in SARS-CoV-2 entry into target cells is the binding of the viral spike
55 protein, S, with the host cell surface receptor angiotensin-converting enzyme 2 (ACE2). Cell
56 tropism is thus expected to be affected by ACE2 expression levels [6-8]. The Omicron spike
57 protein binds soluble human ACE2 strongly [1, 3]. Further, ACE2 is necessary for the entry of
58 all SARS-CoV-2 variants [2-6]. Thus, any variation in ACE2 expression across cell types is
59 likely to have similar effects on the entry efficiencies of all the variants. The cell type-
60 dependent variation in the entry efficiency of the Omicron variant is therefore unlikely to arise
61 from the variations in the ACE2 expression level across cell types. Omicron spike protein
62 incorporation into pseudotyped virus appears to be compromised compared to the Delta and
63 Wuhan D614G strains [5]. However, this reduction in spike protein density is likely to decrease
64 the Omicron entry efficiency across all cell types, thus ruling it out as a potential cause of the
65 differential entry efficiency observed.

66 Following ACE2 engagement, successful entry requires that the spike protein be
67 cleaved into subunits by host proteases, either by the transmembrane serine protease TMPRSS2
68 at the plasma membrane or the cysteine proteases Cathepsin B and Cathepsin L in the

69 endosomal vesicles. The proteases TMPRSS2 and Cathepsin B/L are thought to work
70 independently, facilitating SARS-CoV-2 entry through two independent pathways [8, 9] (Fig.
71 1A). We recently analysed SARS-CoV-2 pseudotyped virus infection of several cell lines [6]
72 using a mathematical model of SARS-CoV-2 entry and found that the relative usage of
73 TMPRSS2 and Cathepsin B/L entry pathways by the original SARS-CoV-2 strain varied
74 widely across cell lines [9]. For example, Vero cells predominantly admitted entry through the
75 Cathepsin B/L pathway, whereas Calu-3 cells allowed entry via the TMPRSS2 pathway. Vero
76 cells overexpressing TMPRSS2 permitted entry via both pathways. Importantly, the original
77 strain displayed the ability to use either pathway, with the preferred pathway possibly based on
78 the relative expression levels of the two proteases [8]. We reasoned that the Omicron variant
79 might use these pathways differently from the original (or the Delta) strain, possibly underlying
80 its different cell tropism.

81 To test this, we extended the above analysis to the recent experiments of Hoffmann *et*
82 *al*. [3] and found a correlation between the Omicron virus entry efficiency and the relative
83 usage of the two entry pathways by the original strain (Fig. 1B). With cell lines (Calu-3 and
84 Caco-2) where the usage of the TMPRSS2 entry pathway by the original strain was dominant,
85 the Omicron pseudotyped virus entry was significantly less efficient than the B.1 and delta
86 strains. By contrast, the Omicron virus entry was more efficient in cell lines (Calu-3 and Caco-
87 2) where the Cathepsin B/L entry pathway usage by the original strain was dominant (Fig. 1B).
88 One possible interpretation of this relationship is that the Omicron variant entry is relatively
89 less effective through the TMPRSS2 pathway and more effective via the Cathepsin B/L
90 pathway than the other strains. Consistent with this notion, camostat mesylate, a TMPRSS2
91 inhibitor, was less potent against the Omicron variant than the Delta variant in blocking live
92 virus infection of VeroE6 cells overexpressing TMPRSS2, which allows entry via both
93 pathways [4]. Moreover, syncytium formation in a cell-cell fusion assay, which requires

94 TMPRSS2 but not Cathepsins, was severely impaired for the Omicron variant compared to
95 both the Delta [4, 5] and the Wuhan-Hu-1 D614G [5] strains. The increased efficiency of
96 Cathepsin B/L usage together with the reduced efficiency of TMPRSS2 usage by the Omicron
97 variant may explain its altered cell tropism relative to the original strain or other variants.

98 To quantify the increased efficiency of the Cathepsin B/L pathway usage by the
99 Omicron variant, we developed a mathematical model and applied it to analyse data from
100 Garcia-Beltran *et al*. [2], who measured the fraction of 293T-ACE2 cells infected by
101 pseudotyped virus bearing the wild-type or the Omicron spike proteins at different viral
102 concentrations (Fig. 1C). We derived an analytical expression to describe infections in the
103 pseudotyped virus infection assay (Supplementary Information, Text S1). Accordingly, the
104 fraction of cells infected, f_a, is given by $f_a = 1 - e^{\frac{k\overline{S_{tc}}}{c_V}(e^{-c_V t_d}-1)V_0}$. Here, V_0 is the initial viral
105 concentration, and $\overline{S_{tc}}$ is the mean susceptibility of the cell population to virus entry,
106 accounting for the usage of both the pathways. (See Text S1 for the description of other model
107 parameters.) Because 293T-ACE2 cells predominantly permit original strain entry via the
108 Cathepsin B/L pathway [9] and given the relatively poor efficiency of TMPRSS2 usage by the
109 Omicron variant (Fig. 1B), we let $\overline{S_{tc}} = \overline{S_c}$, where $\overline{S_c}$ is the mean susceptibility through the
110 Cathepsin B/L pathway. Our model provided good fits to the data (Fig. 1C) and estimated ~4.6-
111 fold increased usage of the Cathepsin pathway by the Omicron variant in 293T-ACE2 cells
112 relative to the original strain (more details in Text S1).

113 The switch in the Omicron variant's preference for entry pathways may have clinical
114 implications. Drugs targeting TMPRSS2, such as camostat mesylate [6], and Cathepsin B/L
115 pathway, such as hydroxychloroquine [10], are in clinical development. The effectiveness of
116 TMPRSS2 inhibitors is likely to decrease and that of Cathepsin B/L inhibitors is likely to
117 increase against the Omicron strain. Furthermore, our mathematical model previously
118 predicted synergy between TMPRSS2 and Cathepsin B/L inhibitors against SARS-CoV-2

infection [9]. Because the Omicron variant's preference for the TMPRSS2 pathway is reduced but not eliminated, combination treatment with TMPRSS2 and Cathepsin B/L inhibitors may exhibit limited but possibly useful synergy against Omicron virus infection of cells that admit both entry pathways. Future studies may establish these implications quantitatively. We suggest that pseudotyped virus infection experiments at different viral concentrations together with inhibitors of TMPRSS2 and Cathepsin B/L pathways, analysed as described here and in Padmanabhan *et al*. [9], might help quantify the relative TMPRSS2 and Cathepsin B/L pathway usage by different strains across cell types including Omicron, potentially explaining their cell tropism and the efficacy of the above treatments.

In summary, our analyses support the notion that the Omicron variant has shifted to better usage of the Cathepsin B/L entry pathway at the expense of the TMPRSS2 pathway. It would be interesting to see how these changes in the entry pathway usage observed in *in vitro* conditions relate to disease severity and transmissibility in animal models and humans.

ACKNOWLEDGEMENTS

We thank Rajat Desikan for help with Figure 1A.

COMPETING INTERESTS

The authors declare that no conflicts of interests exist.

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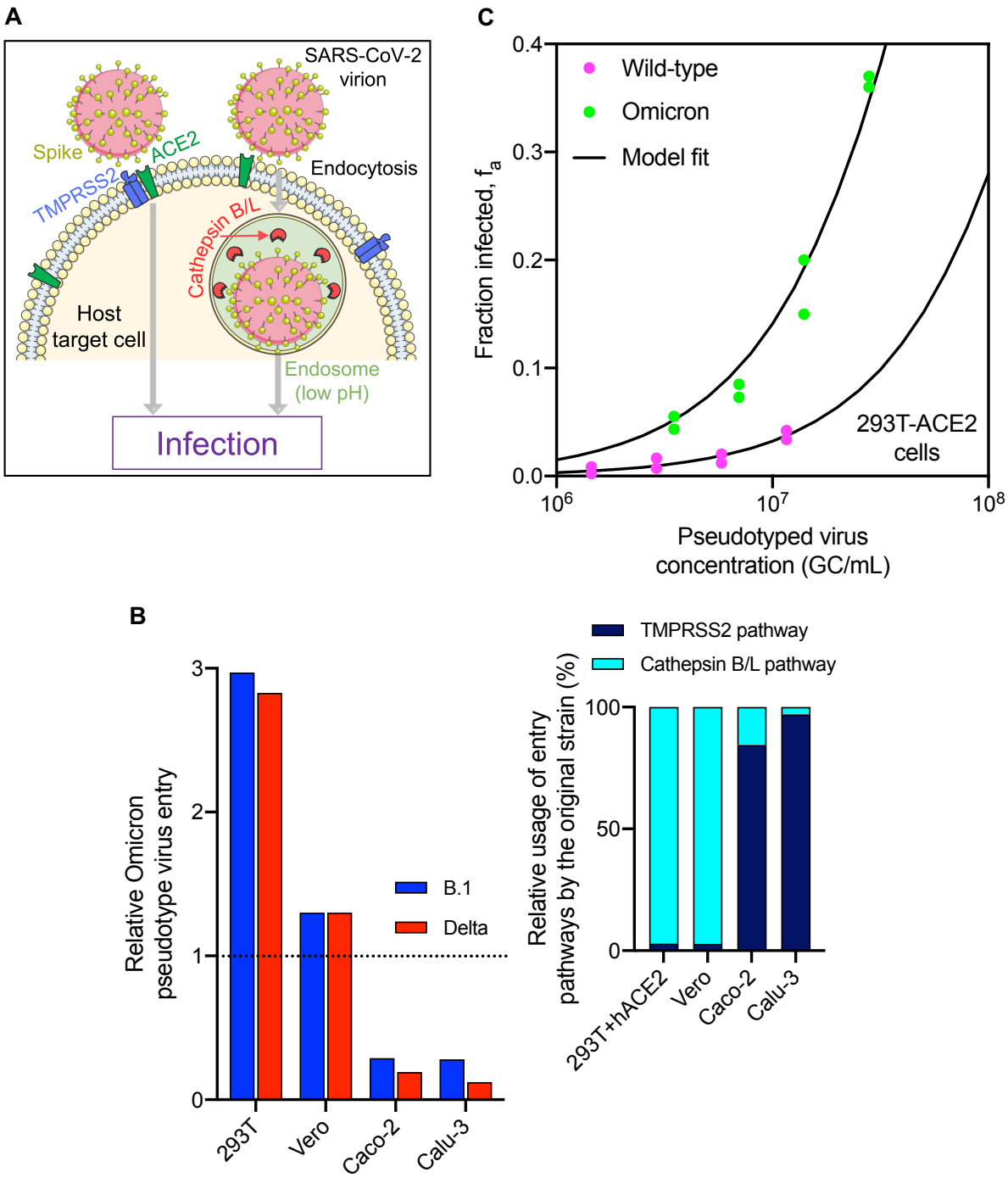

Figure 1. Omicron variant entry efficiency relative to B.1 and Delta strains depends on the relative usage of the TMPRSS2 and Cathepsin B/L pathways. (A) Schematic of the two independent entry pathways accessible to SARS-CoV-2. **(B)** The Omicron entry efficiency relative to either the B.1 (blue) or the Delta (red) variant across cell types. Data was taken from Hoffmann *et al*. [3]. *Inset*, the relative usage of the entry pathways by the original strain. Data was taken from Padmanabhan *et al*. [9]. **(C)** Fits of model predictions (lines) to the

experimental data (filled circles) taken from Garcia-Beltran *et al*. [2] measuring the fraction of cells infected by pseudotyped virus bearing either the wild-type or the Omicron spike proteins. In B and C, experimental data was extracted using Engauge Digitizer 12.1. In C, Data was fit using the tool NLINFIT in MATLAB R2017b.