Replication dynamics and cytotoxicity of SARS-CoV-2 Swedish isolate in commonly used laboratory cell lines

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Running Title: Cellular tropism of SARS-CoV-2

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Abstract:
We assessed the infectivity, replication dynamics and cytopathogenicity of the first Swedish isolate of SARS-CoV-2 in six different cell lines of human origin and compared their growth characteristics. High replication kinetics in absence of cytopathic-effect observed in many cell lines provided important clues on SARS-CoV-2 pathogenesis.

The Study:
The current pandemic of the Coronavirus disease 2019 (COVID-19) caused by the novel coronavirus SARS-CoV-2 is a major global health concern. Presently major research activities have been directed towards developing therapeutics to stop COVID-19[1-5]. Further, in order to understand the biological characteristics of the virus and to identify the drug targets *in vitro* cellular models are used. Vero-E6 is a commonly used cell line for isolation, propagation and antiviral testing since it shows high infectivity, virus production and a prominent cytopathic effect (CPE) upon SARS-CoV-2 infection. However, Vero-E6 originates from monkey and is not a physiologically relevant cell line. Thus, a more suitable cell line of human origin that will be able recapitulate the complexity of host cellular response to the SARS-CoV-2 is warranted. Therefore, in the present study we aimed at determining the SARS-CoV-2 tropism and replication capacity in six different cell lines of human origin that are commonly used in the laboratory.

In a 96 well format, we infected Vero E6 (ATCC#CRL-1586), Calu-3 (ATCC#HTB-55), A549 (ATCC#CCL-185), Caco-2 (CLS-300137), Huh7, 293T and 16HBE and with the first Swedish isolate of SARS-CoV-2 virus (EPI_ISL_411951) with a multiplicity of infection (moi) of 1 and 0.1 as described previously by us[1]. The cellular cytotoxicity was measured by using Viral-ToxGlo assay (Promega) and virus replication was determined in the cell culture supernatant by qPCR targeting the N-gene using Takara PrimeDirect probe, RT-qPCR mix (Takara) starting at 3h post-infection and followed upto 120h[1].

The virus-induced cellular cytotoxicity over time and the viral replication kinetics is shown in Figure 1A and 1B. Infection with moi 0.1 showed better kinetics than moi 1 and attained similar viral-copies at the end of 120h. At moi of 0.1 Vero-E6 showed a complete CPE (viability~3%) by 48h and a significant increase of 3.8 $\log_{10}$ viral-copies by 24h. Of the 6 human cell lines that were tested, Caco2 (intestinal) showed a high infection with a 4.3 $\log_{10}$ copies rise in viral load by 120h at moi 0.1 ($p<0.001$). 293T (kidney;$p<0.01$), Huh7 (liver;$p<0.02$) and Calu-3 (lung;$p<0.05$) showed moderate infection with a change of >1 $\log_{10}$ viral-copies by 120h, while 16HBE (lung) and A549 (lung) showed very poor infectivity with a change of <0.6 $\log_{10}$ viral-copies. It was interesting to note that with moderate infectivity SARS-CoV-2 was able to induce some degree of cytotoxicity in Calu-3 cells (moi 1= 50% and moi 0.1=77%) and none of the other cell lines showed any apparent cytotoxicity (viability>85%).

Since, ACE2 receptor and TMPRSS2 activity have been shown to be critical for SARS-CoV-2 for viral entry into the cell[5, 6]. To correlate the ACE2 and TMPRSS2 expression with the infectivity, we determined the protein expression in the cell lysates by western blot (Figure 1B). Among the infected cell lines, co-expression of ACE2 and TMPRSS2 was most prominent in

Caco2. While Vero-E6 and Calu-3 expressed ACE2, TMPRSS2 expression was very poor. On the other hand Huh7 and 293T lacked ACE2 expression but expressed TMPRSS2 strongly. Lack of correlation between co-expression of the receptors and infectivity indicates individual capacity of the receptors in aiding infection which suggests that there could be alternate mechanisms of cell entry and these need further validation.

Further, to compare the tropism of Swidesh SARS-CoV-2 virus with the other globally isolated strains, we performed a literature survey to determine the infectivity and cytopathogenicity in all the six cell lines as noted above. We have included virus isolates from Germany (FrankfurtFFM1/2020[2] and Muc_IMB1/2020[4]), France (IHUMI2, Ref: PRJEB38023), USA (WA1/2020)[5], Canada (SB3-TYAGNC)[7], China (KMS1/2020)[8], Hong Kong (HKU-001a/2020[3] and VM20001061/2020[9]), Taiwan (NTU01/2020 and NTU02/2020)[10] and Japan (TY-WK-521/2020)[6] and found major differences in cellular tropism and cytopathogenicity. Further, we observed that majority of the strains were able to infect both Caco2 and Calu-3, the Muc_IMB1/2020 strain showed no infectivity in these cell lines[4]. Even the Japanese strain showed no infection of Calu-3[6]. The FFM1/2020 strain showed a rapid CPE in Caco2 by 24h at moi of 0.1[2], while other strains including Swedish isolate did not show any prominent CPE either in higher infective dose or with prolonged time of incubation[3]. Unlike others the Frankfurt strain was isolated and adapted in Caco2 that could be a possible reason for its high CPE[2]. The strain specific differences in infectivity could also be governed by the different infective dose, culture conditions and clonal differences in the cell lines or could be due to structural changes in the virus. Using the Wuhan/Hu-1/2019 strain as reference we interrogated the amino acid changes in the spike protein of these strains. The Frankfurt and Taiwan strains showed no dissimilarities, while the Swedish (F797C), Munich (D614G), China (N74K) and Hong Kong (V367F) strains each showed a single amino acid substitution (Figure 1D). It is interesting to note that the Munich strain containing D614G mutation that became the dominant genotype in Europe shows no infectivity in both Caco2 and Calu-3[4].

In conclusion, our study warrants the need for further understanding of the viral and host factors that govern the infectivity of SAR-CoV-2 in different cell lines and warrants the need for a more physiologically relevant model to study the biological properties of SARS-CoV-2.

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Figure Legends

Figure 1. Infectivity and cytopathogenicity of SARS-CoV-2 in seven different cell lines of human origin. **A.** Indicated cell lines were infected with SARS-CoV-2 at moi of 1 and 0.1 either in duplicate or triplicate. Viral supernatant samples were harvested at 3h post infection (hpi), 24hpi, 48hpi, 72hpi, 96hpi and 120hpi. Viral load was determined by quantitative RT-PCR

targeting the N gene of SARS-CoV-2. The viral load at each time point was compared with baseline viral load at 3hpi (Right panel). The cell viability at each time point was measured by viralToxGlo assay. The viability at each time was determined in comparison to the uninfected control (Left panel). **B.** ACE2 and TMPRSS2 expression in these cell lines were determined by western blot in the cell lysate using specific antibodies (anti-ACE2 E11, SC390851, Santa Cruz; anti-TMPRSS2 H4, sc-515727, santa Cruz and anti-tubulin TUB2.1, T5201, Sigma). **C.** Based on previously published or preprint articles a chart was created showing the infectivity and cytopathogenicity of SARS-CoV-2 strains isolated from different geographical location in the commonly used laboratory cell lines. Boxes are color coded based on intensity as indicated in the figure. The moi used wherever available is mentioned in the bottom. NA= Not available. **D.** Table showing presence of any amino acid substitution in the spike protein of the indicated strains.

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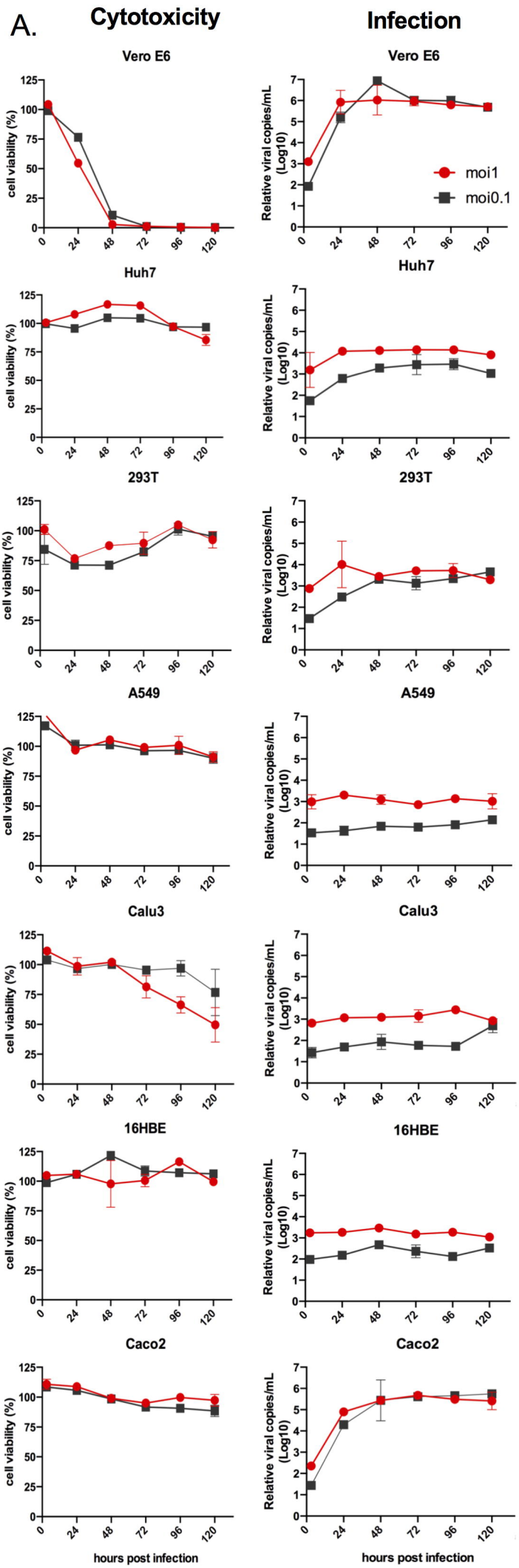

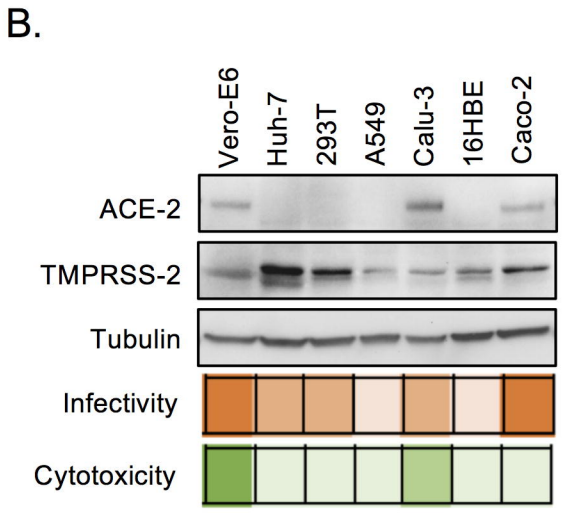

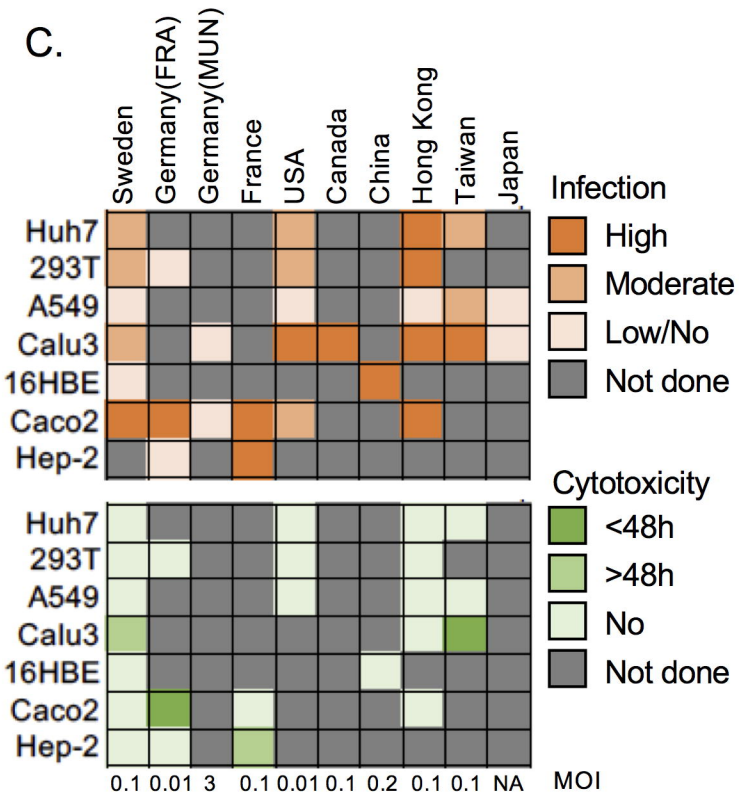

D.

Strain	Spike mutations
Sweden	F797C
Germany FRA	None
Germany MUN	D614G
France	Not available
USA	None
Canada	Not available
China	N74K
Hong Kong	V367F
Taiwan	None
Japan	None