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

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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.

Introduction

In the current pandemic of Coronavirus disease 2019 (COVID-19), caused by the novel Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), numerous research activities have been focusing on better understanding the mechanisms of viral pathogenesis as well as developing effective antivirals against the infection(*1–5*). In this scenario, Vero-E6 cell line has been widely employed for viral isolation, propagation and antiviral testing, due to its high infectivity, virus production and a prominent cytopathic effect (CPE) upon infection. However, since Vero-E6 originates from monkey, a more physiologically relevant cell line of human origin is warranted, that is better able to recapitulate the complexity of host cellular response to the SARS-CoV-2.

In this study, we aimed at determining the SARS-CoV-2 tropism and replication capacity in six different human cell lines that are commonly used in laboratories. Our data provide a comprehensive view on how SARS-CoV-2 behaves differently in different human cell lines depending on the viral strain and, possibly, the isolation method. Overall, our work represents a useful tool for choosing a suitable cell system to investigate the viral pathogenicity and drug susceptibility.

The Study

We infected Vero-E6 (ATCC#CRL-1586), Calu-3 (ATCC#HTB-55), A549 (ATCC#CCL-185), Caco2 (CLS-300137), Huh7, 293T and 16HBE with the first Swedish isolate of SARS-CoV-2 virus (SWE/01/2020) at a multiplicity of infection (moi) of 1 and 0.1 as previously described(*1*). Virus-induced cytotoxicity was evaluated by measuring the cellular ATP using Viral-ToxGlo™

assay (Promega) and virus replication kinetics were determined in the cell culture supernatant by qPCR targeting the N-gene(*6*) using PrimeDirect™ Probe RT-qPCR mix (Takara) starting at 3h post-infection (hpi) and followed up to 120hpi(*1*). As shown in Figure 1A, infection with moi 0.1 showed better kinetics than moi 1 and attained similar viral-copies at 120hpi. At moi 0.1 Vero-E6 showed a complete CPE (viability<3%) by 48hpi and a significant increase of 3.8 log10 viral-copies at 24hpi. Of the six human cell lines that were tested, Caco2 (intestinal) showed highest infection with a 4.3 log10 copies rise in viral load at 120hpi, with 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 log10 viral-copies at 120hpi, while 16HBE (lung) and A549 (lung) showed very poor infectivity with a change of <0.6 log10 viral-copies. Interestingly, viral-induced cytotoxicity was only observed in Calu-3 cells (viability at moi 1= 50% and moi 0.1=77%) while none of the other cell lines showed any apparent cytotoxicity (viability>85%) (Figure 1B).

ACE2 receptor and TMPRSS2 activity has been shown to be critical for SARS-CoV-2 entry into the cell(*5*,*7*). To correlate the ACE2 and TMPRSS2 expression with the infectivity, we determined the protein expression in the cell lysates by western blot (Figure 2A). Among the infected cell lines, co-expression of ACE2 and TMPRSS2 was most prominent in Caco2, followed by Vero-E6 and Calu-3 that showed poor TMPRSS2 expression. Contrarily, Huh7 and 293T strongly expressed TMPRSS2 but lacked ACE2 expression. Our results showed no strong correlation between co-expression of the receptors and infectivity, indicating that each receptor has an individual role in aiding the infection.

Finally, to compare the tropism of Swedish 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

(FFM1/2020(*2*) and Mun_IMB1/2020(*4*)), France (IHUMI2) (*8*), USA (WA1/2020)(*5*), Canada (SB3-TYAGNC)(*9*), China (KMS1/2020)(*10*), Hong Kong (HKU-001a/2020(*3*) and VM20001061/2020(*11*), Taiwan (NTU01/2020 and NTU02/2020)(*12*) and Japan (TY-WK-521/2020)(*7*) and found major differences in cellular tropism and cytopathogenicity (Figure 2B). Specifically, we observed that the majority of the strains were able to infect both Caco2 and Calu-3, except for the Muc_IMB1/2020 (no infectivity in both the cell lines)(*4*) and the Japanese strain (no infectivity in Calu-3)(*7*). The Frankfurt_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 at higher infective dose or with prolonged time of incubation (*3*). Lastly, 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 2C).

Conclusion

Establishing SARS-CoV-2 infectivity and cytopathogenicity in a human cell line is key to testing new antivirals as well as understanding viral-host interplay in a more physiologically relevant setting. Several recent studies testing different local strains, show conflicting results concerning infectivity and cytopathogenicity in human cell lines (Figure 2B), especially when Caco2 and Calu-3 cells are employed. Particularly, the Swedish isolate tested here showed high infectivity in Caco2 and moderate infectivity for Calu-3, not aligning with any particular strain. A stark difference was also noted in cytopathogenicity, wherever reported. In our study, upon

infection, Calu-3 stopped growing, rounded up and mottled, as compared to the uninfected control. The morphological change observed in Calu-3 might represent a cellular mechanism to control viral infection and therefore needs more investigation. In Caco2 we did not observe any appreciable cytopathogenicity as was reported for the French(*8*) and the Honk Kong strain(*3*). However, the Frankfurt strain caused rapid CPE in Caco2 within 24hpi. Unlike others, the Frankfurt strain was the only strain that was isolated and adapted in Caco2 that could have possibly lead to higher infectivity and CPE of this strain in the cell line(*2*). In addition, different infective doses, structural changes in the virus, culture conditions and clonal differences in the cell lines, could also govern strain specific differences in infectivity.

A major role in viral infectivity is played by envelope glycoproteins and cellular receptors, which initiate the early phases of the viral life cycle. Therefore, we first investigated whether mutations in the spike glycoproteins of the different strains presented above could affect their infectivity and cytopathogenicity. However, no major amino acid changes was observed in the spike protein, except for the Munich strain harboring D614G mutation that had become the dominant genotype in Europe and considered to be more infectious in humans(*13*). Interestingly, this strain shows no infectivity in both Caco2 and Calu-3(*4*).We then correlated the viral infectivity with the expression of ACE2 and TMPRSS2 proteins that are considered essential for viral entry. Our results showed no strong correlation between co-expression of the receptors and infectivity and suggest that while ACE2 expression is important for the virus infectivity, increased expression of TMPRSS2 alone can enhance virus uptake into the cell(*14*). However, we cannot exclude the possibility that other molecules or endocytosis mechanisms are involved in viral recognition and entry. In addition, in the present study, we have investigated the replicative capacity of the virus by measuring the newly produced virions in the culture supernatant. In different cell lines there

might be other mechanisms that interfere with viral replication following entry, that require further validation.

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

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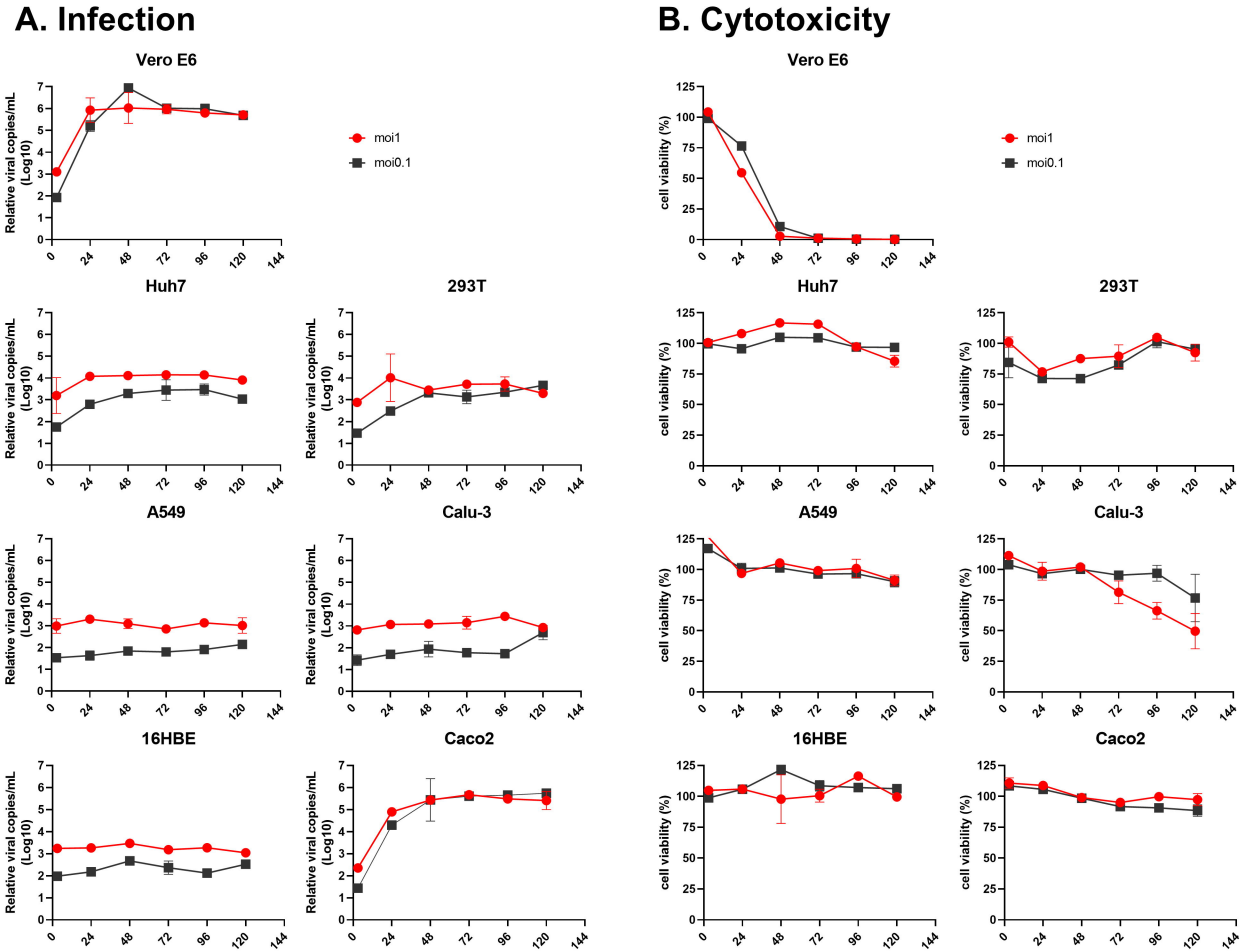
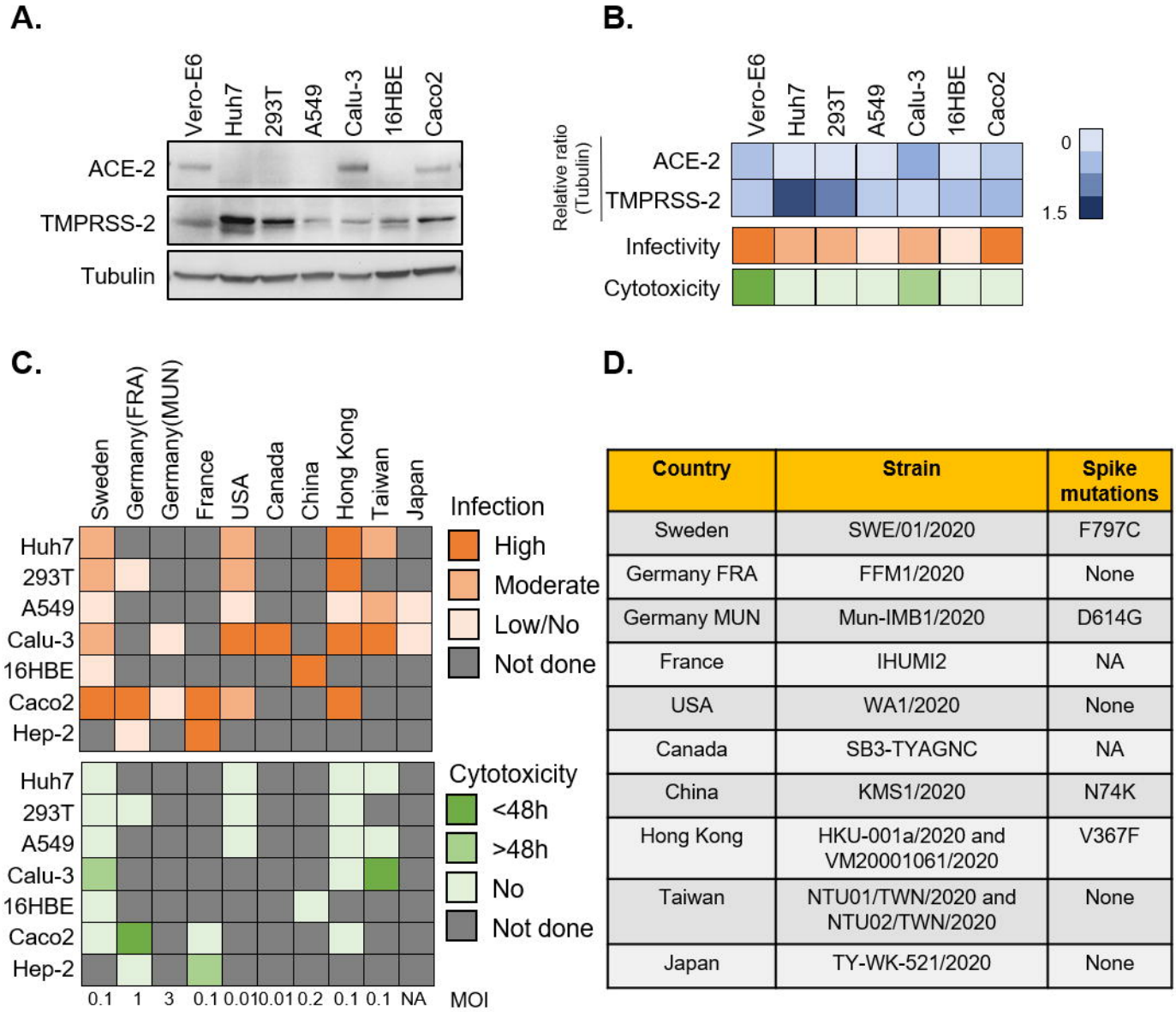
Figure Legends:

Figure 1. Infectivity and cytopathogenicity of SARS-CoV-2 in Vero E6 and six 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. **B)** The cell viability at each time point was measured by Viral-ToxGlo™ assay. The viability at each time was determined in comparison to the uninfected control.

Figure 2. Determinants of SARS-CoV-2 infectivity A) ACE2 and TMPRSS2 expression in these cell lines were determined by western blot in the cell lysate. **B)** Heatmaps with relative protein quantification, infectivity and cytotoxicity are shown. For infectivity and cytotoxicity, refer to color legends in Figure 2C. **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. **D)** Table showing presence of any amino acid substitution in the spike protein of the indicated strains. NA= Not available.


A. Infection
B. Cytotoxicity
Vero E6
Huh7
293T
A549
Calu-3
16HBE
Caco2
moi1
moi0.1
Relative viral copies/mL (Log10)
cell viability (%)
0
24
48
72
96
120
144



Country	Strain	Spike mutations
Sweden	SWE/01/2020	F797C
Germany FRA	FFM1/2020	None
Germany MUN	Mun-IMB1/2020	D614G
France	IHUMI2	NA
USA	WA1/2020	None
Canada	SB3-TYAGNC	NA
China	KMS1/2020	N74K
Hong Kong	HKU-001a/2020 and VM20001061/2020	V367F
Taiwan	NTU01/TWN/2020 and NTU02/TWN/2020	None
Japan	TY-WK-521/2020	None