SARS-CoV-2 Permissive glioblastoma cell line for high throughput antiviral screening

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1.簡述論文的概要與重大發現

雖然 SARS-COVID 目前有疫苗可以預防，也有抗病毒藥物可以使用，但變異種(例：Omicron...)仍繼續四處傳播，因此作者想研發出更有效的抗病毒藥物篩選平台，但從以前的經驗來看，篩選藥物庫需要依賴非常強大的細胞感染系統。目前用於 SARS-COVID 藥物篩選的細胞株 Vero E6 其實是比較不適合的，因此作者將 U87 cell 和 ACE2 結合成一種 U87.ACE2+ cell，使此細胞能和 SARS-COVID 的 Spike protein 結合，有效的感染細胞。為了想驗證 U87.ACE2+ cell 是否能有效結合病毒，作者先將此細胞株和以 Spike transfect 的 HEK293T cell 融合，來證明 U87.ACE2+ cell 能有效和 Spike domain 接合。

接著觀察被 SARS-COVID 感染後的 U87.ACE2+ cell 所導致的 CPE 及 N protein 的表現量。

作者觀察到不論是將 MOI 數值提高或培養的時間拉長，被感染的 U87.ACE2+ cells 對野生型的 SARS-COVID 有 highly permissive，同時也可誘發廣泛的 CPE。比起 Vero E6 cell，受感染的 U87.ACE2+ cell 可以更快速的引起 CPE 及細胞裂解，具有強大的細胞感染能力，且對抗病毒藥物 Remdesivir、Nirmatrelvir 、Molnupiravir 的抑制都有很強的感受性。

2.對論文內容的疑問

在 Western-blotting 的結果中可以看到 U87.ACE2+ cell 中的 Cathepsin L(glycosylated mature CTSL)也有明顯的表現，但並沒有提到為什麼會比原生的 U87 cell 多。

為什麼不選用本身就具有 ACE2 和 TMPRSS2 的 Calu-3 細胞株，而是選擇將 ACE2 transduct 到本身不具有 ACE2 的 U87 cell 上。

3.論文的缺點與評論

只用了三種抗病毒藥物來證實被 SARS-COVID 感染的 U87.ACE2+ cell 可以有效的被抑制，但對於其他抗病毒藥物是否一樣能發揮有效的抑制性有待討論。

在主要的地方有放入影片及補充可以讓讀者更清楚了解細胞病變或是抑制的過程。

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ABSTRACT

Despite the great success of the administered vaccines against SARS-CoV-2, the virus can still spread, as evidenced by the current circulation of the highly contagious Omicron variant. This emphasizes the additional need to develop effective antiviral countermeasures. In the context of early preclinical studies for antiviral assessment, robust cellular infection systems are required to screen drug libraries. In this study, we reported the implementation of a human glioblastoma cell line, stably expressing ACE2, in a SARS-CoV-2 cytopathic effect (CPE) reduction assay. These glioblastoma cells, designated as U87.ACE2$^+$, expressed ACE2 and cathepsin B abundantly, but had low cellular levels of TMPRSS2 and cathepsin L. The U87.ACE2$^+$ cells fused highly efficiently and quickly with SARS-CoV-2 spike expressing cells. Furthermore, upon infection with SARS-CoV-2 wild-type virus, the U87.ACE2$^+$ cells displayed rapidly a clear CPE that resulted in complete cell lysis and destruction of the cell monolayer. By means of several readouts we showed that the U87.ACE2$^+$ cells actively replicate SARS-CoV-2. Interestingly, the U87.ACE2$^+$ cells could be successfully implemented in an MTS-based colorimetric CPE reduction assay, providing IC_{50} values for Remdesivir and Nirmatrelvir in the (low) nanomolar range. Lastly, the U87.ACE2$^+$ cells were consistently permissive to all tested SARS-CoV-2 variants of concern, including the current Omicron variant. Thus, ACE2 expressing glioblastoma cells are highly permissive to SARS-CoV-2 with productive viral replication and with the induction of a strong CPE that can be utilized in high-throughput screening platforms.

1. Introduction

Undoubtedly, the current coronavirus disease 2019 (COVID-19) pandemic has not only posed a serious threat to the international health, but it has also impacted people's daily lifestyle and work, and the global economy. Since its outbreak in December 2019, SARS-CoV-2, the causative agent of COVID-19, has quickly spread around the world, leading to over 400 million confirmed cases and more than 5.8 million deaths worldwide as of February 11, 2022 (https://covid19.who.int/). Despite the great success of the current vaccines against SARS-CoV-2, we still cannot control the spread of new variants and/or prevent re-infections. This emphasizes the urgent need to develop effective antiviral countermeasures.

Major efforts are ongoing to develop novel therapeutics for COVID-19 treatment and/or effective prophylactic approaches to prevent viral spread (Yadav et al., 2021). In the context of early preclinical studies for SARS-CoV-2 antiviral assessment, screening platforms for drug libraries rely on the use of robust cellular infection systems, mostly based on immortalized cell lines originating from respiratory, but most often non-respiratory, tissues (Murgolo et al., 2021; Uemura et al., 2021; Chiu et al., 2022; Ramirez et al., 2021; Grau-Exposito et al., 2022; Ko et al., 2021; Chu et al., 2020). In fact, many initial screenings for SARS-CoV-2

Abbreviations: ACE2, Angiotensin-Converting Enzyme 2; CC, cytotoxic concentration; COVID-19, coronavirus disease 2019; CPE, cytopathic effect; CTS, cathepsin; MFI, mean fluorescence intensity; MOI, multiplicity of infection; OD, optical density; RdRp, RNA dependent RNA polymerase; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S, Spike; TMPRSS2, Transmembrane protease, serine 2.

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