6-17-2018

Characteristics of rVSV-ZEBOV production kinetics in HEK293 and Vero cells

Sascha Kiesslich
McGill University, Canada, sascha.kiesslich@mail.mcgill.ca

Amine Kamen
McGill University, Canada

Jean-François Gélinas
McGill University, Canada

Rénald Gilbert
National Research Council, Montreal, Canada

Follow this and additional works at: http://dc.engconfintl.org/vt_vii

Part of the Engineering Commons

Recommended Citation

This Abstract and Presentation is brought to you for free and open access by the Proceedings at ECI Digital Archives. It has been accepted for inclusion in Vaccine Technology VII by an authorized administrator of ECI Digital Archives. For more information, please contact franco@bepress.com.
Characteristics of rVSV-ZEBOV production kinetics in HEK293 and Vero cells

Sascha Kiesslich¹, Jean-François Gélinas¹, Xavier Eliseeff², Rénéal Gilbert², Amine Kamen¹

¹ McGill University, Montreal, Canada, ² National Research Council, Montreal, Canada

Introduction

The vesicular stomatitis virus (VSV) can be used as an effective vaccine platform, inducing both cellular and humoral immunity. Since VSV infections of humans are mostly asymptomatic, recombinant VSV (rVSV) can be used as a platform to safely deliver and express foreign antigens. This research study focused on cell culture production of an rVSV expressing the Ebola virus glycoprotein on its surface (rVSV-ZEBOV).

Limited data is available in the literature about the growth characteristics of this virus during the production process. In our study, we investigated the influence of several process parameters on the viral titer of rVSV-ZEBOV produced in the Vero cell line and in a suspension-adapted HEK293-based cell line without serum.

Methods

rVSV-ZEBOV production in Vero

Figure 1: Schematic of VSV WT (A) expressing the VSV-G glycoprotein (yellow) and rVSV-ZEBOV (B) expressing the Ebola virus glycoprotein (pink).

Figure 2: Outline of rVSV expression development.

Figure 3: Production yields of infections with rVSV-ZEBOV at MOI 0.01, 0.001, and 0.0001 of adherent Vero cells in 24-well plates at 37°C. Samples were harvested at indicated timepoints and titers were measured by TCID50 and ddPCR. Infectious virus titers were determined in triplicates. The highest titer (6.64×10^7 TCID50/mL) was observed after 60 hours post infection at MOI 0.001 with a rate of 2.36 VG/MP.

Figure 4: Production yields of infections with rVSV-ZEBOV at MOI 0.01 of adherent Vero cells in 24-well plates at 3 different temperatures (31°C, 34°C, and 37°C). Samples were harvested at indicated timepoints and titers were measured by TCID50 and ddPCR. The highest titer (3.16×10^8 TCID50/mL) was observed after 72 hours post infection at 34°C with a rate of 179 VG/MF.

rVSV-ZEBOV incubation at different temperatures

Figure 5: Production yields of infections with rVSV-ZEBOV at MOI 0.001 of adherent Vero cells in 24-well plates at 3 different temperatures (31°C, 34°C, and 37°C). Samples were harvested at indicated timepoints and titers were measured by TCID50 and ddPCR. The highest titer (1.32×10^7 TCID50/mL) was observed after 60 hours post infection with a rate of 2.08 VG/MP.

rVSV-ZEBOV stability

Figure 6: Production yields of rVSV-ZEBOV infection at MOI 0.001 of HEK293 at 2 different temperatures (34°C and 37°C) in 24-well plates with 1×10^5 cells/mL in 2 mL per well in suspension culture. Titers were measured by TCID50. Bars represent the mean of triplicate production studies a standard deviation. There was a statistically significant difference (p = 0.0012) between the two group means as determined by unequal two-tailed t test indicating that production at 34°C was higher than at 37°C.

Figure 7: Titers of rVSV-ZEBOV exposed to different temperatures for increasing amounts of time. Titers were measured by TCID50. Bars represent the mean of triplicate production studies a standard deviation. At 1 week incubation, there were statistically significant differences between group means as determined by one-way ANOVA (F (4,16) = 8.14, p = 0.0002) followed by Dunnett’s post-test for lower titer for incubations at temperatures 34°C and above when compared to incubation at 4°C.

Conclusions

- MOI affects rVSV-ZEBOV production kinetics in both Vero and HEK293 (data not shown) with a lower MOI resulting in a delayed peak of virus production.
- Typically, during the course of production, the infectious titer reaches a plateau after which it starts to decline. In contrast, the number of viral genomes continues to increase. This effect can be attributed to a loss of viral infectivity over time whereas the total viral particles count increases.
- Production of rVSV-ZEBOV at 34°C results in the highest infectious titers for both cell lines. This might result from the weak rVSV-ZEBOV thermosensitivity at higher temperatures as shown in figure 7. Consequently, rVSV-ZEBOV production should be carried out at 34°C to achieve higher infectious titers and to reduce the observed loss of infectivity. In contrast, freeze-thaw cycles seem to have a lesser impact on viral stability.
- Operation at higher MOIs might contribute to improve the ratio of infectious to total particles, by reducing the process production timelines and enabling earlier virus harvest.
- In addition, this study showed the proof of principle for the production of rVSV-ZEBOV in serum-free suspension cultures of HEK 293 and Vero cells to enable more streamlined process development and scale-up and take advantage of most advanced cell culture technologies.

- Overall, the results indicate significant potential to improve manufacturing of the VSV-vectored Ebola vaccine in order to meet global health needs.

Acknowledgement

The rVSV-ZEBOV was kindly provided by G. Kobinger (Université Laval, Quebec, ON, Canada).

Source of plasticware images: Saniver Medical Art (smart.saniver.com)
Source of VSV schematics: ViralZone, SIB Swiss Institute of Bioinformatics (www.expasy.org/viralzone)