In Vivo Gene Transduction via Lentiviral Retargeting System

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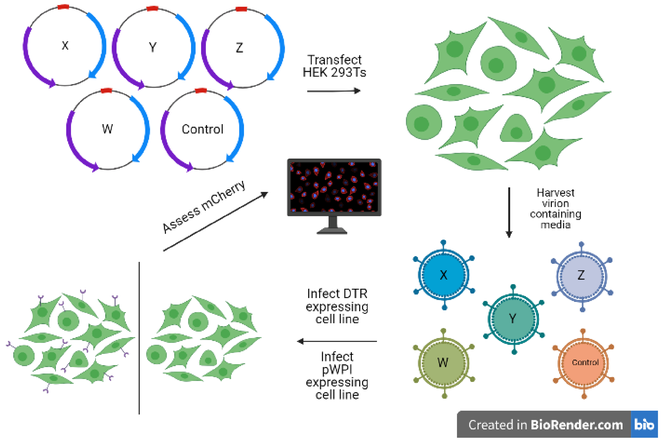
Abstract



Introduction

- There is a plethora of chronic diseases such as coronary artery disease (CAD) and other neurological & physiological diseases that have high mortality rates, yet there are few therapeutic options or targets to lessen the burden of such devastating diseases.
- In the early 2000s, Harold Varmus and colleagues designed a mouse expressing the avian receptor TVA and demonstrated effective and specific transduction in vivo using avian retroviruses[1], but because these retroviruses can only infect actively dividing cells, this model had limited applicability model[3].
- Lentiviral vectors have become attractive candidates for gene delivery within the last two decades as they are capable of efficiently delivering genes in both dividing and nondividing cells [4].
- Several groups have demonstrated that lentivirus can be successfully pseudotyped with a 'blinded' Sindbis virus envelope protein and have shown successful re-targeting of these pseudotyped particles by attaching various cell-specific binding proteins to an external loop of the Sindbis envelope protein[1],[4].

Materials and Methods

- Construction of a stable diphtheria toxin receptor (DTR) expressing HEK 293T cell line
- Pseudotype lentivirus with Sindbis-DT-RBD fusion envelope proteins
- Transduce DTR-expressing cells with Sindbis-DT-RBD pseudotyped lentivirus

For alternate experiment:

- Construction of a stable diphtheria toxin receptor (DTR)-SpyCatcher expressing HEK293T cell line
- Pseudotype lentivirus with pMD2.G-VSVG-SpyTag fusion envelope proteins
- Transduce DTR-SpyCatcher expressing cells with pMD2.G-VSVG-SpyTag pseudotyped lentivirus

Results

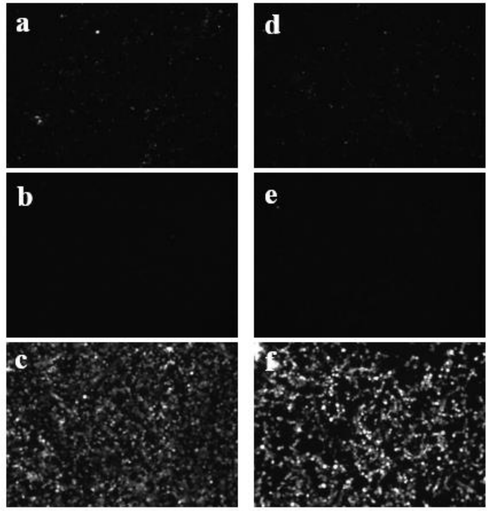


Figure 1. Experimental lentiviral constructs insufficient in robustly mediating the transduction of control and experimental cell lines. a-c are PWPI-expressing cells and d-f are DTR-expressing cells. a & d were infected with sindbis-DT-RBD virus. b & e were infected with sindbis-DTRBD-3XFlagTag with polylinkers lentivirus. c & f were infected with traditional VSV-G pseudotyped lentivirus.

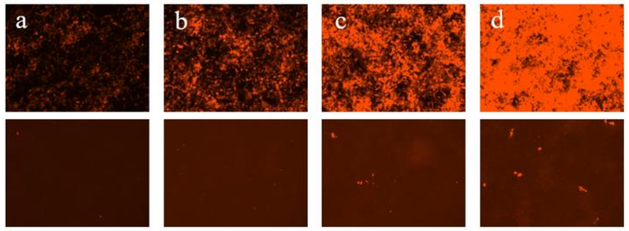


Figure 2. SpyTag-SpyCatcher interaction marginally mediates infection of HEK293T cells. Fluorescent microscopy images of infected cells expressing mcherry after (a) 30 min, (b) 1 hr, (c) 6 hrs, and (d) 24 hrs. The top row contains the control and the bottom the experimental.

Conclusions

- The data suggests the proposed lentiviral constructs and the interaction between SpyCatcher and SpyTag were insufficient in mediating a stronger infection than the traditional VSV-G pseudotyped lentivirus positive control.
- The proposed envelope protein iterations are not viable for in vivo experimentation.
- The data; however, suggests the design of the viral envelope protein is significant and influences the mediation of infection.
- Continued work is needed to design other iterations of the viral envelope protein capable of mediating gene transfer in a cell-type specific manner

References

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