Testing the Efficiency of a HA Pseudotyped Lentiviral Vector for Treating Cystic Fibrosis Lung Disease

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Introduction:

Gene therapy has the potential to prevent the onset or progression of Cystic Fibrosis (CF) airway disease. We have developed a unique dosing technique using a lentiviral (LV) vector with the VSV-G pseudotype shown to target receptors on the basolateral surface of airway cells. This study is designed to examine the gene transfer levels that result after altering the envelope to an influenza hemagglutinin (HA) pseudotype, known to primarily target receptors on the apical epithelial surface. We have carried out *in vitro* experiments to test the effectiveness of the HA pseudotyped LV vector carrying either the LacZ or Luciferase (Luc) reporter genes.

Methods:

VSV-G and HA pseudotyped HIV-1 LV vectors containing LacZ or Luc were produced by $CaCl_2$ precipitation. The supernatant was collected and stored at -80º C. To test vector effectiveness 293T cells were plated onto 6 well plates at a density of 0.125 x 10^6 cells/ml per well and aliquots of each vector (n = 3) were added to the cells.

Untreated plated cells were used as controls. Titres and expression levels were assessed by counting LacZ expressing cells, or by bioluminescence imaging (BLI) (Xenogen IVIS-100), as appropriate.

Results:

The titre of the unconcentrated VSV-G supernatant was 1.38 x 10^6 TU/ml (6.6 x 10^4 (SD)), while the titre of the HA supernatant was 1.09 x 10^5 TU/ml (8.5 x 10^3 (SD)) (Figure 1)

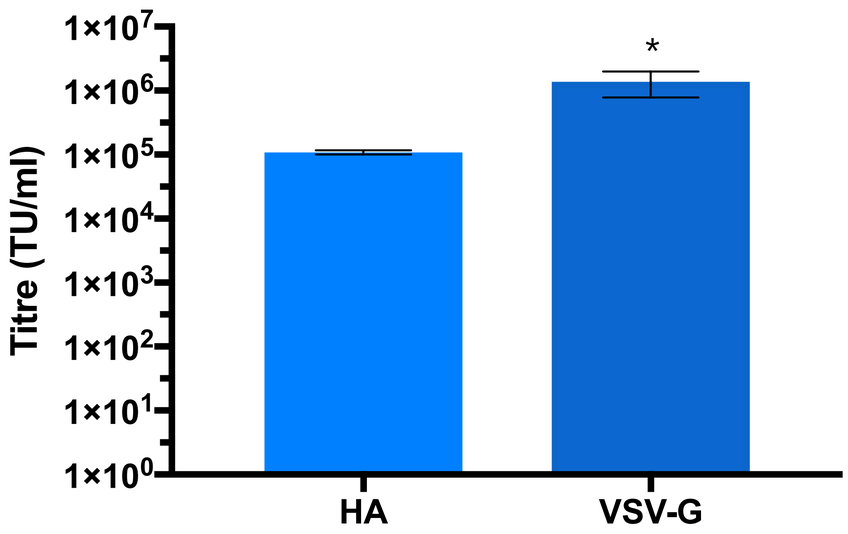

Figure 1: Unconcentrated titre for HA and VSV-G pseudotyped HIV-1 LV vectors. (*p<0.05, Mann Whitney Test, n = 3)

The VSV-G-Luc expression was 5.96 x 10^6 photons/sec (1.95 x 10^6 (SD)), and for HA -Luc vector was 4.23 x 10^4 photons/sec (1.38 x 10^5 (SD)) (Figure 2).

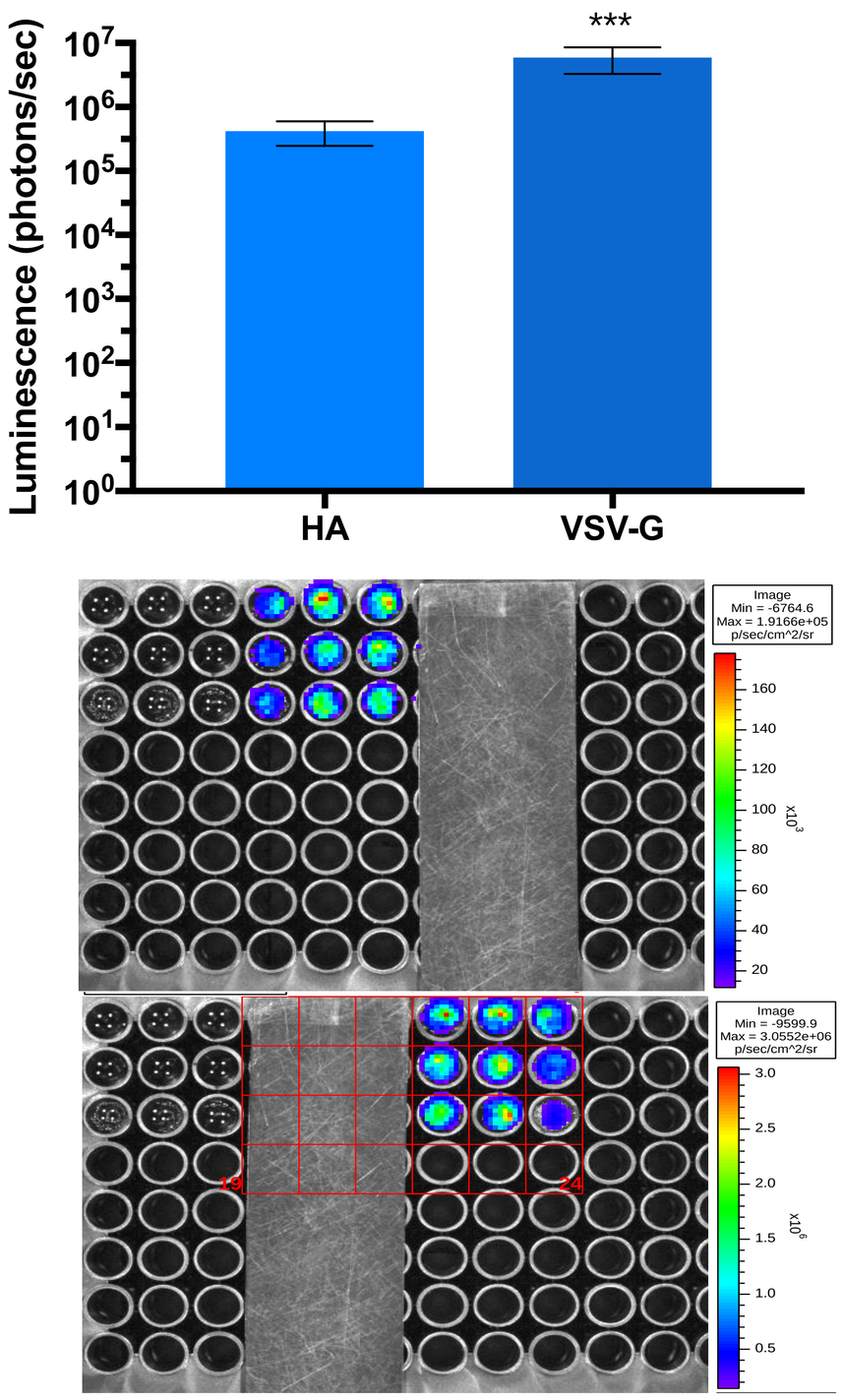

Figure 2: In culture bioluminescence levels (photons/sec) of 293T cells transfected with the HA and VSV-G pseudotyped HIV-1 LV vectors. (***p<0.0002, unpaired t-test with Welch's correction, n = 3)

Conclusion:

Using identical production conditions resulted in a much higher titre for the vector pseudotyped with VSV-G envelope than the HA vector. This has substantial practical implications for animal studies where high titres are essential for producing strong and persistent transduction.

Future Studies:

Future experiments will begin shortly to compare gene expression levels produced by both vectors in a rodent animal model.

A short-term study will ustilise the LacZ reporter gene to assess initial levels of cell transduction with the use of an airway conditioning compound (LPC), while a long-term study ustilising the Luc reporter gene will assess the level of basal cell transduction produced by HA.

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