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Article *in* Molecular Therapy · May 2005

DOI: 10.1016/j.ymthe.2005.07.022

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lentiviruses, and vectors derived from them. At least part of this blockade is thought to be due to restriction factors, which act to subvert incoming virions down non-replicative pathways. At high multiplicities of infection (MOI) such factors can be titrated away, allowing the cell to be transduced. Elsewhere it has been demonstrated that, similarly to HIV and MLV, Equine Infectious Anaemia virus (EIAV) vectors may also be restricted in a variety of cell types, including human cells. If true, this may have implications regarding EIAV vectors' utility for human gene therapy applications. We have developed highly engineered completely minimal EIAV vectors, and have demonstrated the vectors' potential in a number of pre-clinical models of human disease, including recent studies in models of Parkinson's disease and amyotrophic lateral sclerosis (ALS). We are currently developing the vector system and an associated bank of safety tests, as a prerequisite for treating Parkinson's disease in patients.

The work presented here shows analysis of the dynamics of transduction of human/animal cells by EIAV vectors pseudotyped with different envelope glycoprotein isoforms, to assess the degree of restriction of EIAV vectors in human cells. Through these studies, we have found that two envelope glycoprotein isoforms have markedly different properties from each other when used to pseudotype EIAV GFP-vectors. These envelopes result in significantly different transduction efficiency of a panel of human cells; between 10 to 100-fold improvement in transduction was observed over a range of MOIs. Using the same vector preparations, the transduction efficiency of HEK293T and D17 (canine) cells was indistinguishable. The data indicates that although EIAV may be restricted in certain cells at low MOIs (<2), the major factor in the differential transduction efficiencies observed was the envelope glycoprotein isoform employed. Therefore, by choosing the appropriate envelope glycoprotein, it is unlikely that host cell restriction will be a barrier to successful human gene therapy using EIAV vectors.

Interestingly, these data indicate that the choice of envelope protein employed to pseudotype a retro/lentiviral vector may lead to subversion of certain restriction pathways. This may be due to the manner in which the virion first enters the cytoplasm i.e. at plasma membrane or via endosomes. Therefore, events occurring at the time of cell entry may be vital in determining the fate of an incoming virion from other vector systems.

We will present data describing the effect of alternative psuedotyping EIAV vectors on transduction efficiency. These data indicate that (1) choice of envelope glycoprotein can have a marked effect on transduction efficiency of human cells by EIAV vectors, and consequently (2), empirical titration of retro/lentiviral vectors on more clinically relevant cells may give more meaningful estimates of vector strength in respect to the clinical application.

Work has been funded by industry (Biomedica), and there are no unresolved issues with presenting this work.

482. Neuronal Gene Transfer with HIV-1-Based Lentiviral Vectors Pseudotyped with Lyssavirus Glycoproteins

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Background: The delivery of therapeutic proteins to the central nervous system represents an appealing strategy for the treatment of nerve injury and disorders of the CNS. Important factors determining CNS targeting include tropism of the viral vectors and retrograde transport of the vector particles. In the present study, an HIV-1-based lentiviral vector was pseudotyped with four different envelope (Env) glycoproteins generating four different HIV-1 pseudotypes. To select a high tropism HIV-1 pseudotype for CNS targeting, SHSY-5Y neuroblastoma cells were infected with pseudotyped vectors.

Methods: To pseudotype HIV-1 vectors, four different Env expression plasmids were constructed using RNA extracted from BHK-21 cells infected with members of the *Lyssavirus* genus including rabies virus, European bat lyssavirus, Lagos bat virus and Duvenhage virus. HIV-1-based lentiviral vectors bearing the *E. coli* β-galactosidase (LacZ) gene controlled by the CMV-IE promoter were subsequently pseudotyped with four different lyssavirus-derived glycoproteins. Theses HIV-1 pseudotypes were screened in SHSY-5Y neuroblastoma cells to determine their degree of neuronal tropism *in vitro*. After infection for 72 hours, infected cells were stained with X-Gal and β-galactosidase-positive cells were counted.

Results: At a multiplicity of infection (MOI) of 1 (BHK-21 units), rabies pseudotypes infected 36.89% of the SHSY-5Y cells (+/- 1.052 SEM). Duvenhage pseudotypes infected 13.134% of the cells (+/- 1.142 SEM). European bat pseudotypes infected 3.171% of the cells (+/- 0.351 SEM) and Lagos bat pseudotypes infected 2.79% of the cells (+/- 0.0935 SEM). The SHSY-5Y cells infected at an MOI of 0.1 exhibited similar levels of infection.

Conclusion: Our *in vitro* study demonstrated that HIV-1-based lentivirus vectors could be pseudotyped with glycoproteins from rabies virus, European bat lyssavirus, Lagos bat virus and Duvenhage virus to generate infectious HIV-1 vector particles. The rabies glycoprotein-pseudotyped HIV-1 vector possessed the highest neuronal tropism among the four HIV-1 pseudotypes tested *in vitro*. An *in vivo* study involving intranerve injection of these pseudotypes is underway to test the retrograde transport efficiency of HIV-1 pseudotypes for CNS targeting through peripheral injection.

Acknowledgement: This work was supported by grants from NINDS KO8 NS43305, and ALSA.

483. Targeted Receptor Trafficking Affects the Efficiency of Retrovirus Transduction

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We describe the development of an experimental system to test the hypothesis that the efficiency of retrovirus transduction is dependent on the pathway of virus entry into the host cell and the intracellular trafficking itinerary of the cellular receptor with which it interacts. The experimental system consists of three model target cell lines, derived from HeLa cells, that stably express one of three interleukin-2 receptor alpha chain (CD25) chimeras – TAC, TAC-CD16, and TAC-DKQTLL - that have identical extracellular domains but different intracellular trafficking itineraries, and a targeted amphotropic murine leukemia retrovirus whose envelope proteins were modified to include a binding site for TAC at their N-termini. We found that the efficiency of retrovirus transduction was affected by the distribution and trafficking itinerary of the TAC receptors. Transduction of cells that expressed TAC-DKQTLL was nearly 4-fold lower than transduction of control cells that did not express any of the TAC receptors. In contrast, transduction of cells that expressed TAC was 1.6 fold higher than transduction of control cells, whereas transduction was not significantly affected by the expression of TAC-CD16. Our results suggest that in the course of designing a targeted retrovirus it may be prudent to target only those receptors that internalize retroviruses via pathways that most efficiently support post-binding steps of infection.