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Poster presentation

P05-01. Generation of a HI-viral packaging cell line as scaffolding for a lentiviral display system

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Background

Lentiviruses with their ability to produce high virus titers and to efficiently infect undividing mammalian cells represent an ideal platform for the display and directed evolution of envelope proteins. A lentiviral display enables the presentation of envelope protein complexes in a natural membrane environment both with a correct folding pattern and oligomerisation.

Methods

Therefore, we developed a reciprocal system of a full-length HIV packaging cell line and a shortened HI-viral shuttle vector. The Tat-deficient packaging construct is stably integrated in a VSVG-pseudotyped cell line and transactivated by the shuttle vector by expression of TATGFP from a heterologous promoter which also indicates positive cells. Expression of the HI-viral structure proteins is driven by a natural 5' LTR promoter and a 3' polyadenylation signal, whereas the envelope expression out of the shuttle vector is achieved by an IRES. This system of two vectors is tightly controllable and bypasses the problem of limited packaging capacity into HIV-1 virions.

Results

Transactivation of the packaging construct by the shuttle vector resulted in particle release at 80% of wt HIV level. We observed only 13% incorporation of packaging construct RNA in viral particles but a highly efficient packaging of the shuttle vector RNA. Replication of the shuttle vector in the complementing packaging cell line resulted in titers up to 1,1 × 10^7 IU/ml after ultracentrifugation, and efficient infection of mammalian cells could be achieved with resulting virus. Furthermore, we were able to show a high transgene envelope expression which was even increased relative to expression driven by the natural Env ORF.

Conclusion

This vector system offers a platform for the viral display of envelope antigens in a highly controllable manner and will be used to affinity-mature envelope antigens by the use of neutralizing antibodies against HIV-1.