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Approaching a Tat-Rev Independent HIV-1 Clone towards a Model for Research

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Abstract : Introduction: Human Immunodeficiency Virus type 1 (HIV-1) is responsible for the acquired immunodeficiency syndrome (AIDS), a leading cause of death worldwide infecting millions of people each year. Despite intensive research in vaccine development, therapies against HIV-1 infection are not curative, and the huge genetic variability of HIV-1 challenges to drug development. Current animal models for HIV-1 research present important limitations, impairing the progress of in vivo approaches. Macaques require a CD8+ depletion to progress to AIDS, and the maintenance cost is high. Mice are a cheaper alternative but need to be 'humanized,' and breeding is not possible. The development of an HIV-1 clone able to replicate in mice is a challenging proposal. The lack of human co-factors in mice impedes the function of the HIV-1 accessory proteins, Tat and Rev, hampering HIV-1 replication. However, Tat and Rev function can be replaced by constitutive/chimeric promoters, codon-optimized proteins and the constitutive transport element (CTE), generating a novel HIV-1 clone able to replicate in mice without disrupting the amino acid sequence of the virus. By minimally manipulating the genomic 'identity' of the virus, we propose the generation of an HIV-1 clone able to replicate in mice to assist in antiviral drug development. Methods: i) Plasmid construction: The chimeric promoters and CTE copies were cloned by PCR using lentiviral vectors as templates (pCGSW and pSIV-MPCG). Tat mutants were generated from replication competent HIV-1 plasmids (NHG and NL4-3). ii) Infectivity assays: Retroviral vectors were generated by transfection of human 293T cells and murine NIH 3T3 cells. Virus titre was determined by flow cytometry measuring GFP expression. Human B-cells (AA-2) and Hela cells (TZMbl) were used for infectivity assays. iii) Protein analysis: Tat protein expression was determined by TZMbl assay and HIV-1 capsid by western blot. Results: We have determined that NIH 3T3 cells are able to generate HIV-1 particles. However, they are not infectious, and further analysis needs to be performed. Codon-optimized HIV-1 constructs are efficiently made in 293T cells in a Tat and Rev independent manner and capable of packaging a competent genome in trans. CSGW is capable of generating infectious particles in the absence of Tat and Rev in human cells when 4 copies of the CTE are placed preceding the 3’LTR. HIV-1 Tat mutant clones encoding different promoters are functional during the first cycle of replication when Tat is added in trans. Conclusion: Our findings suggest that the development of an HIV-1 Tat-Rev independent clone is challenging but achievable aim. However, further investigations need to be developed prior presenting our HIV-1 clone as a candidate model for research.

Keywords : codon-optimized, constitutive transport element, HIV-1, long terminal repeats, research model
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