Effect of lentiviral vector-mediated transduction on the tight junction integrity of polarized airway epithelial cells

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Introduction The mechanism(s) of viral vector interaction with the apical plasma membrane and internalization has been an intensely studied question. Lentiviral (LV) vectors are promising agents for gene therapy of genetically determined pulmonary diseases, such as cystic fibrosis, but no studies have tested directly the effect of LV vectors on tight junction (TJ) stability and organization in airway epithelial cells. In this work, we investigated the interaction between the vesicular stomatitis virus G glycoprotein (VSV-G)-pseudotyped HIV-1-derived LV vector expressing GFP and polarized 16HBE41o-cell line. In particular, we evaluated the transduction efficiency as well as acute cytotoxicity, transepithelial resistance (TER) and occludin localization at the TJ level.

Materials and Methods 16HBE41o- cells were grown as a polarized sheet of cells and then incubated with the LV vector at different multiplicities of infection (MOI) for 4 or 24 hours and with the polycation branched 25 kDa polyethylenimine (PEI) associated to LV to increase LV efficiency. The cells were either immediately studied for propidium iodide (PI) staining, by flow cytometry analysis, and cell viability, by MTT assay, or incubated for further 48 hours for evaluation of GFP expression. The evaluation of GFP expression was carried out by flow cytometry. For occludin immunolocalization, polarized 16HBE14o-cells were processed for intracellular staining with FITC-conjugated mouse anti-occludin antibody.

Results and Discussion We have observed an higher percentage of GFP-positive cells at MOIs 500 and 2000, although these high viral loads showed to be cytotoxic, as assessed by increase in PI staining and decrease in cell viability, and harmful for the epithelial tightness, as demonstrated by the decrease of TER and delocalization of occludin from the TJs.

Transduction of cells with PEI/LV particles resulted in 2.5-3.6 fold increase of percentage of GFP positive cells only at the highest PEI:LV ratio (1 X 10^7 PEI molecules/transducing units with 50 MOI LV) as compared to plain LV. At this dose PEI/LV transduction resulted in 6.5 ± 2.4% of PI-positive cells. On the other hand, PEI/LV particles did not determine any alteration of TER and occludin localization. We conclude that PEI may be useful for improving the efficiency of gene transfer mediated by LV vectors in airway epithelial cells, without high acute cytotoxicity and alteration in epithelial tightness.

Key words
Occludin