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Lentiviral vector pseudotyped with Sendai virus F and HN proteins uses sialylated glycan receptors to efficiently target human airway cells.

Rosie J Munday[1], Tiziana Coradin[2], Rachael Nimmo[2], Yatish Lad[2] and Kyriacos Mitrophanous[2], Stephen C Hyde[1] and Deborah R Gill[1].
[1]Radcliffe Department of Medicine, University of Oxford, Oxford, UK.
[2] Oxford Biomedica (UK) Ltd, Oxford, UK.

We are developing a lentiviral vector pseudotyped with the F and HN coat proteins from Sendai virus (Murine Respirovirus) in order to target lung cells. The F/HN pseudotype was shown to facilitate efficient transduction of a range of cells in the murine lung (Alton et al. 2017 Thorax 72:137). Whereas Sendai virus receptor targeting utilises various subtypes of ubiquitous sialylated glycans, it is not yet known how well this targeting will translate to the human lung, due to differences in the distribution of these subtypes between species. In collaboration with Oxford BioMedica, two alternative influenza-based pseudotypes, predicted to differ in sialylated glycan subtype targeting, have been developed for comparison with F/HN. Here, we investigate transduction efficiency and cell type targeting by pseudotyped HIV-based vectors in human airway air-liquid interface (hALI) cultures derived from human Bronchial Epithelial Cells (hBEC) to assess dependency on sialylated glycan receptors.

Human ALI cultures were treated 6-8 weeks post airlift with equivalent doses of recombinant HIV vectors expressing eGFP, pseudotyped with glycoproteins from Sendai virus (F/HN), influenza virus (HA/NA), or Vesicular Stomatitis Virus (VSVg) as a negative control. After 14 days, transduced hALI cultures were imaged and the percentage area of eGFP fluorescence above background calculated. At a dose of 7.5e7 Transducing Units (TU) the F/HN pseudotype resulted in high levels of eGFP (18.5 - 51.6% area eGFP; n=4) whereas VSVg pseudotyped vector showed no transduction (0-0.8% area eGFP; n=4), presumably due to lack of access to basolateral VSVg receptors. The F/HN vector was significantly more efficient at transducing hALI cultures (7.5e6 TU; n=4) than either of the HA/NA influenza-based pseudotypes tested (Kruskal-Wallis, F/HN vs HA/NA, $P=0.0333$ and $p<0.0001$), with stable EGFP expression persisting for more than 4 months.

Specific cell-type targeting was determined in hALI cryosections with colocalization of native eGFP fluorescence and antibodies against β-Tubulin (ciliated cells), Mucin 5Ac (goblet cells), and Cytokeratin 5 (basal cells); the F/HN, and both HA/NA Influenza pseudotypes, were confirmed to target all three cell types. Consistent with previous published literature, no club cells (Uteroglobin antibody) were detected in the hALI cultures derived from hBEC (n=4). Lectins specific for α2,3 and α2,6 subtypes of glycan sialylation were used to stain for the receptor distribution in hALI cultures, revealing an abundant distribution of α2,6 (and a subtype of α2,3) in hALI cultures (n=4), representative of the distribution in human airways described in the literature. To confirm that F/HN and HA/NA pseudotyped vector cell entry is dependent on sialylated glycans, hALI cultures were pre-treated with sialidase to cleave sialic acid, prior to vector administration. Transduction, quantified by native eGFP fluorescence, was significantly reduced for all F/HN and HA/NA vectors (Kruskal-Wallis, Sialidase pre-treatment vs no pre-treatment, $p<0.0001$ (F/HN), $P=0.0194$ and $P=0.0022$ (HA/NA); n=4).

These data confirm the distribution and importance of sialylated glycans in the efficient transduction of airway cells, and studies are underway to characterise the specific sialylated glycans responsible for targeting of hALI cultures. These data should allow us to predict whether these pseudotyped vectors are likely to be efficient in the human airways during clinical studies.