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A method for assessing the distribution of eGFP expressing cells in the mouse lung following lentiviral vector transduction.

Background:

The UK Cystic Fibrosis gene therapy consortium have developed a Lentiviral vector pseudotyped with the F and HN coat proteins from Sendai virus which successfully targets a range of cell types in the lung (Alton et al. 2017)(1). So far, the distribution and quantification of transduced cells in murine models has been characterised using immunohistochemistry, which is an indirect method of staining.

In this study, we aimed to use native eGFP fluorescence to identify eGFP-expressing cells.

Method:

Mice were dosed with either 1e8 or 3e8 transducing units (TU) of rSIV.F/HN.hCEF.eGFP (n=4 per dose); and lung lobes were fixed, cryoprotected and cryosectioned on day 7 or 28 post-administration. Imaging the cryosections using the EVOS FL Auto 2 imaging system (Thermo Fisher), capable of 'stitching' captured fields of view together, the entire lung lobe could be captured. In order to minimise autofluorescence quantification, ImageJ software was employed to calculate the percentage area of epithelium or parenchymal tissue above a threshold fluorescence for regions of interest.

Results:

Airway epithelial fluorescence values in treated mice were significantly higher (Mann-Whitney $p < 0.0001$) than those from naïve mice, indicating that this method is a useful measure of eGFP expression. Approximately 40% of the airway epithelium in both dosed groups exceeded the fluorescence threshold, whereas in epithelia from naïve mice the median percentage area above threshold was zero. Using this method, the percentage of the epithelium transduced with lentiviral vectors can be readily quantified, and the extent of transduction throughout the whole lung can be quickly visualised.

1. Alton EFWF, Beekman JM, Boyd AC, Brand J, Carlon MS, Connolly MM, et al. Preparation for a first-in-man lentivirus trial in patients with cystic fibrosis. *Thorax* [Internet]. 2017 Feb 16 [cited 2017 Dec 1];72(2):137–47. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27852956>