SINGLE CELL ASSAYS FOR QUANTIFYING MRNA AND PROTEIN DURING CYSTIC FIBROSIS GENE THERAPY TRIALS

Authors: Anthony J Sinadinos[1,4], Ana Sergijenko[1,4], Aarash D Saleh[1,4], Kyriel M Pineault[1,4], Neda AM Nafchi[1,4], Jack W Hickmott[1,4], Tushar R Choudhary[2,4], Catherine L Mclaughlin[2,4], Toby Gamlen[3,4], Deborah R Gill[3,4], Stephen C. Hyde[3,4], Gerry McLachlan[2,4], Eric WFW Alton[1,4*] and Uta Griesenbach[1,4*]

Presenting Author is underlined

[1] Gene Therapy Group, National Heart and Lung Institute, Imperial College London, London, UK

[2] The Roslin Institute and R(D)SVS, University of Edinburgh, Edinburgh, UK

[3] Nuffield Division of Clinical Laboratory Sciences, Radcliffe Department of Medicine, University of Oxford, Oxford, UK

[4]UK CF Gene Therapy Consortium

[*]Joint Senior Authors

Introduction: Gene therapy for cystic fibrosis is approaching clinical trials. Using a lentiviral vector pseudotyped with the fusion and hemagglutinin-neuraminidase proteins from Sendai virus, the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene can be inserted into airway cells leading to the long-term production of functional CFTR protein in animal models. To evaluate the success of a therapy in the clinic, methods are needed that can accurately and sensitively measure RNA and protein from limited human clinical trial samples. Here we develop RNAscope, reverse transcriptase droplet digital PCR (RT-ddPCR), and digital proximity ligation assay (dPLA) techniques that can be used to detect and quantify lentivirus RNA and Enhanced Green Fluorescent Protein (EGFP) from single cells of tissues transduced in vitro, ex vivo, and in vivo.

Methods: HEK293T, A549, human air liquid interface cultures (ALI), mouse nasal brushings, mouse airway, human nasal epithelial, and piglet airway epithelial cells were transduced with lentivirus encoding EGFP and the woodchuck hepatitis post-transcriptional regulatory element (WPRE). Following transduction, cells were spun and fixed onto slides for RNAscope using a probe against WPRE. Alternatively, transduced cells were single-cell flow-sorted into 96-well plates. RNA and protein were measured from the same cell. For RNA quantification, one-step RT-ddPCR was performed with a probe against WPRE. For protein quantification, dPLA used oligonucleotide conjugated antibodies against GFP.

Results: RNAscope successfully stained for WPRE in A549, mouse airway cells, and human nasal brushings. In mouse airways cells, RNAscope measured a median of 33.9% (interquartile range (IQR): 23.9 – 43.8%) and median of 24.9% (IQR: 9.3 – 29.8%) of WPRE+ cells seven and 28 days after transduction, respectively. RT-ddPCR successfully detected WPRE containing RNA from all tissues analysed: single HEK293T, human ALI, mouse nasal epithelial cells, and piglet airway epithelial cells. In mouse nasal brushings, RT-ddPCR measured a median of 951.0 WPRE RNA copies/cell (IQR: 810.8 – 1170.0 WPRE RNA copies/cell), and dPLA measured a median of 1.3×10^7 EGFP molecules/cell (IQR: 9.7×10^6 – 1.6×10^7 EGFP molecules/cell) 28 days after transduction. Additionally, EGFP protein levels significantly correlated to EGFP fluorescence levels measured during cell sorting ($R^2 = 0.94$, $p < 0.0001$).

Conclusions: RNAScope, RT-ddPCR, and dPLA can successfully detect lentiviral RNA and EGFP from multiple tissue sources. These methods could prove useful for testing therapeutic vector transduction efficiency both in pre-clinical studies and clinical trials.