Lobe-specific targeting of lentiviral vector gene transfer to the lungs of adult rats

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Introduction:

- CF rat models are the smallest animal model of CF lung disease resulting from mutations in the CFTR gene. Their small size, manageable pathology, and the abundance of resources available for rodents, makes them an ideal model for the assessment of pre-clinical therapies for CF.
- The small size of the rat lung has made selective and specific dosing to small regions or lobes difficult. We have therefore designed and implemented the first reliable targeting of a gene transfer vector to specific small regions of the rat lung.
- Here, we describe the setup for performing this minimally invasive technique, and show successful delivery of VSV-G pseudotyped lentivirus to selected lung lobes.

Methods:

- The bronchoscope system includes a Storz rigid bronchoscope (1.1 mm diameter; Model 11582A), a miniature LED light source, and a USB camera. A miniature screen can be used to display the live bronchoscopic image, and/or a standard computer used to display and record the procedure (Fig 1).
- For gene transfer, anaesthetised rats were held upright by hooking their dorsal incisors over a stiff wire loop on a custom-made stand (Fig 2a).
- The bronchoscope tip was navigated to a pre-selected region of the lung using a schematic of the rat tracheobronchial tree (Figure 2b), past the vocal cords, through the trachea (Fig 2c) and past the carina (Fig 2d), using the live video as guidance.
- The pre-selected lobes were first treated with 0.1% lysophosphatidylcholine, then the same region was re-treated one hour later with a lentiviral vector expressing the LacZ marker gene.

Results:

- The bronchoscope tip could be placed at least as far as the 4th branch of the airway in 200 gram rats.
- Using the bronchoscope for performing gene vector instillations allows fluid to be predominantly targeted to individual lobes, including the left lung (Fig 3a), the right accessory lobe (Fig 3b), the right superior lobe (Fig 3c), and the right middle lobe (Fig 3d).
- Histological assessment revealed transduction of ciliated epithelial cells, as well as type I and II pneumocytes, and macrophages (Data not shown).

Conclusions:

- The delivery of fluids to the rodent lung has typically been performed relatively blindly, through the nose, or the trachea via an orally or surgically placed intubation tube, resulting in high levels of variability between animals, and a range of off target effects.
- The bronchoscopic gene delivery method we report is a significant advance in rodent lung dose delivery, and we have begun gene vector dosing studies in CF rats. The same methods used to deliver gene transfer vectors can also be applied to localised induction of disease, for example with viral or bacterial pathogens, and bacteria-embedded agar beads.

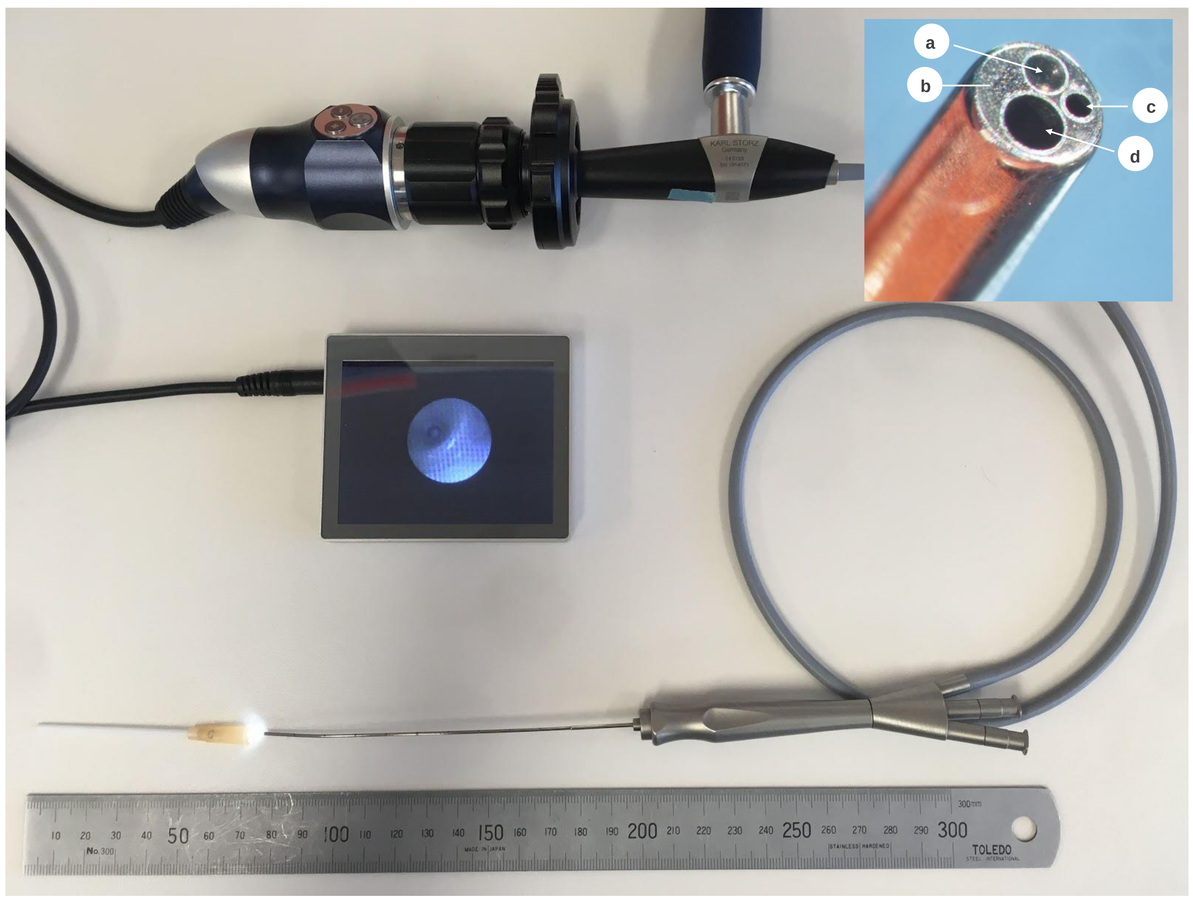

Figure 1: Rodent bronchoscopy and fluid delivery system. The inset shows the tip depicting (a) camera fibres, (b) illumination fibres (c) 0.2 mm irrigation channel, and (d) and 0.45 mm working channel.

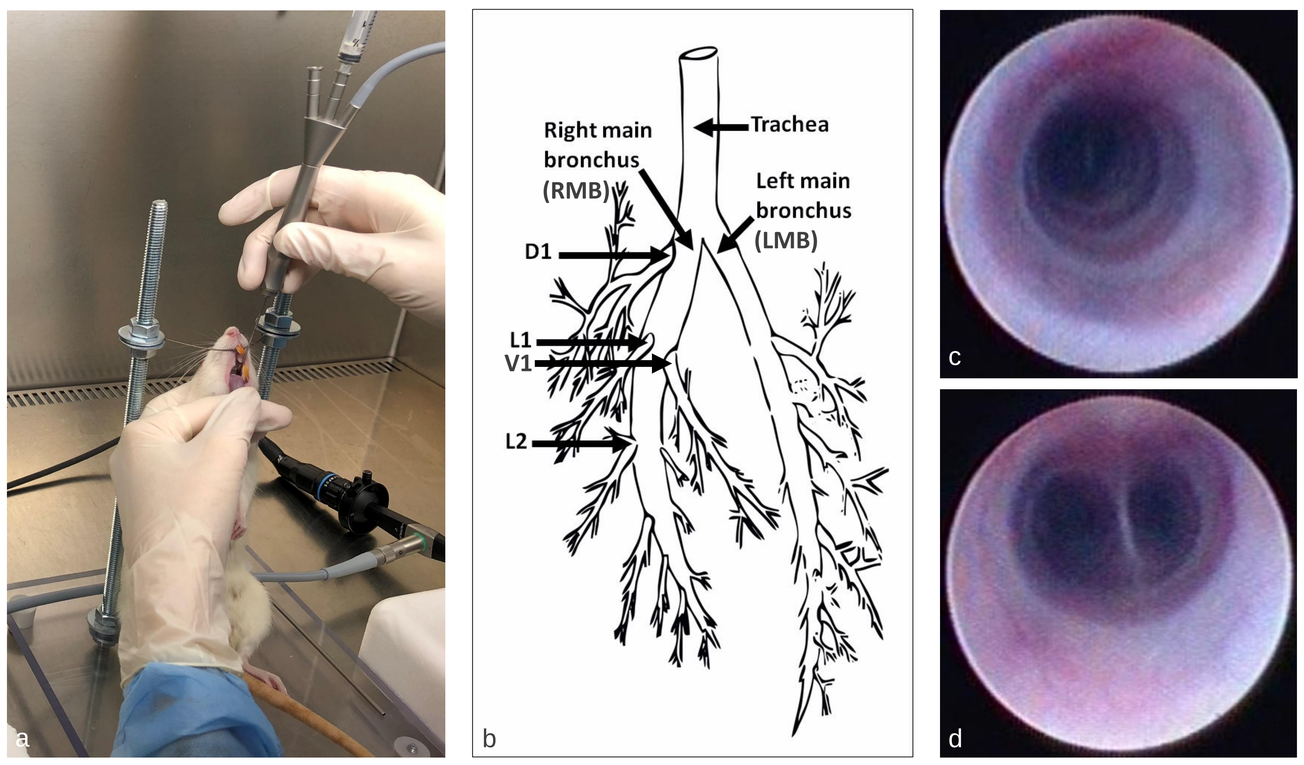

Figure 2: (a) LV vector dosing procedure performed in BSII cabinet with rat attached to stand; (b) diagram of the rat bronchial tree (from Monteiro 2014); (c) bronchoscope image from the trachea; (d) image from RMB L1.

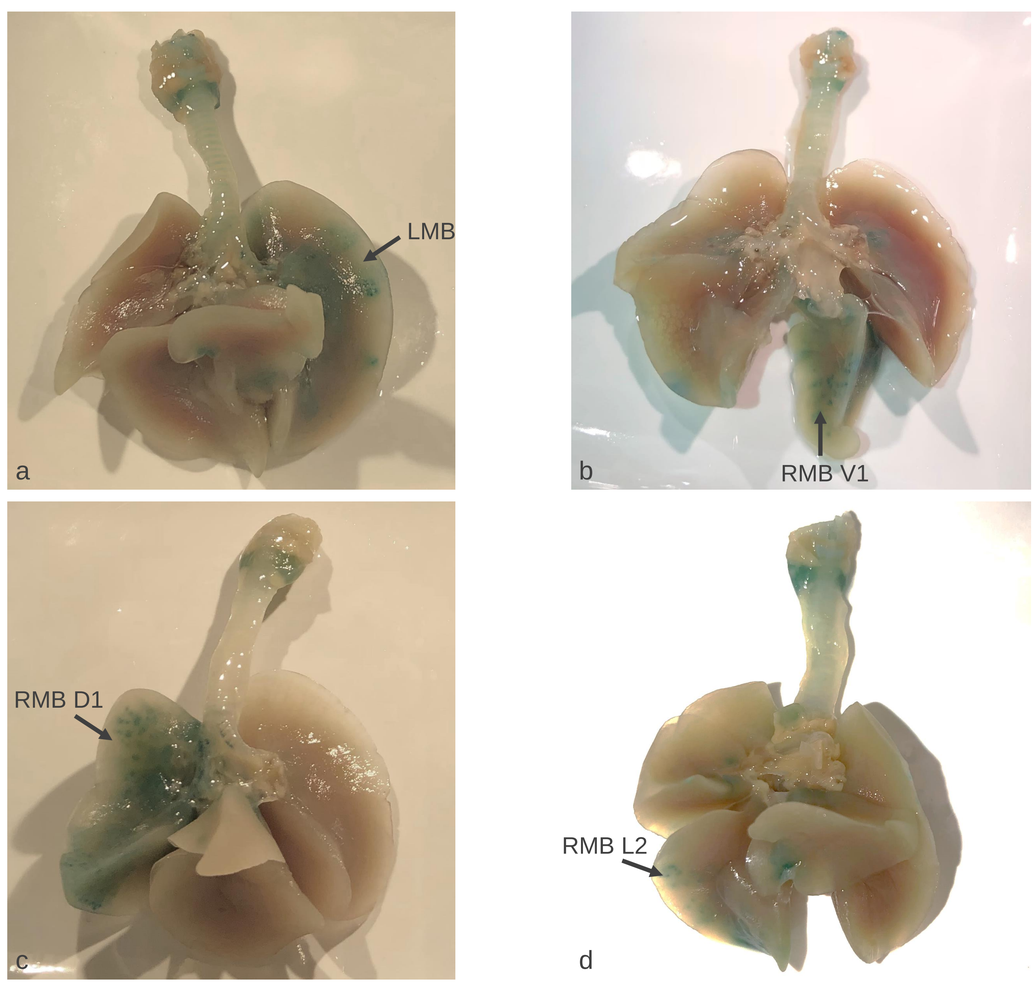

Figure 3: LacZ reporter gene expression enables identification of blue transduced cells in individually targeted lobes, including: (a) the left lung; (b) the right accessory lobe; (c) the right superior lobe; (d) the right middle lobe.

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