Epithelial Disruption Enables Human Airway Stem Cell Transplantation in Mouse Airway Airways

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Introduction:
• Airway disease is a primary cause of morbidity and early mortality for patients with cystic fibrosis (CF).
• Cell transplantation therapy has proven successful for treating some immune disorders and this approach has potential to correct the airway disease phenotype associated with CF.

Aim:
• Assess whether conditioning the mouse nasal airway with the agent polidocanol (PDOC) to disrupt the epithelial layer enables transplantation of cultured human airway basal cells (hABC).

Methods:
• Normal HBEC (LONZA, USA: CC-25450S) were seeded onto collagen-coated flasks to isolate and proliferate hABC. To track cells following transplantation they were treated with a lentiviral vector (MOI 10) containing the luciferase (Luc) gene.
• To prepare the nasal airways for cell transplantation the airway epithelium was exposed to 2% PDOC, to disrupt the epithelial cell layer. Normal female C57Bl/6 mice received 4 µl of either PBS (control (n=10)) or 2% PDOC (n=10) into the right nostril.
• Two, or twenty four hours later, three 10 µl aliquots of gene-transduced cells (hABC-Luc) were delivered to the same nostril of those mice. Another group of mice (n=3) were culled at 2 or 24 hours after PDOC delivery to assess the impact on the epithelium using keratin 5 (Krt5) and Haematoxylin and Eosin (H & E) analysis (Fig 1).
• Bioluminescence imaging (BLI; Xenogen, IVIS) was performed at 1, 3, 5 and 8 weeks post-dosing to assess hABC transplantation via luminescence of Luc-expressing cells.

Results:
• Analysis of mouse nasal airways 2 or 24 hours after PDOC delivery showed the dislodgment of epithelial cells was more severe at the later time point. Two hours after PDOC treatment the epithelium was partially removed and the basal cell compartment was in tact. Twenty four hours after PDOC treatment the epithelium removal was more comprehensive and portions of the basal cell population had been dislodged with the basement membrane also being compromised (Fig 1).

Conclusions:
• Three mice that received hABC-Luc cell transplantation 2 hours after PDOC treatment did not survive the procedure.
• Three of the seven remaining mice showed significant luminescence for up to 3 weeks (p<0.01, RM ANOVA vs PBS control) as detected by BLI (Fig 2). Luciferase gene expression declined by the 5 week time point, and was below detectable levels by 8 weeks.
• In control animals (PBS conditioned) no luciferase expression was detected by BLI at any time point (Fig 2 & 3a).

Conclusion:
• These encouraging findings show that epithelial disruption produced by the PDOC airway conditioning procedure can produce initial and persisting Luc-expressing cell transplantation in the nasal airways.
• Future studies will need to assess the potential for the protocols presented here in both autologous and allogeneic cell transplantation into CF disease animal models using functional assays to determine the therapeutic value of the procedure.

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Figure 1: Histological images of the anterior mouse nose 2 hours or 24 hours after PDOC treatment only; the septum (NS) separates the treated (Left) and untreated control (Right, solid black arrowheads) nasal airways. Regions of epithelial cell loss (black arrows), the presence of cell debris (open arrows), a loss of basal cells and basal lamina integrity (open arrowheads) are apparent. Scale bar 0.2 mm. Panels b and c are an enlargement of a; e and f are an enlargement of d. Panels a,b,d and e all H&E stained; c and f are Krt5 stained serial sections from b and e, respectively.