



ENaC Knockdown in the Mouse Lung Using siRNA

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Overview

- Lung pathology in individuals with Cystic Fibrosis (CF) is linked to sodium hyper-absorption.
- The defective regulation of the epithelial sodium channel ENaC is thought to be a major contributor to reduced Airway Surface Liquid (ASL) volume and impaired mucociliary clearance of the CF airways.
- Strategies designed to inhibit ENaC function may result in clinical benefit.
- We investigated the possibility of using siRNA to reduce expression of ENaC in the mouse lung, as a strategy to reduce sodium hyper-absorption in the CF lung.

Identification of Potent Anti-ENaC siRNA Molecules

The epithelial sodium channel ENaC contains three homologous but distinct subunits termed alpha, beta and gamma ENaC. The functional channel is thought to comprise two alpha subunits complexed with a single beta and gamma subunit.

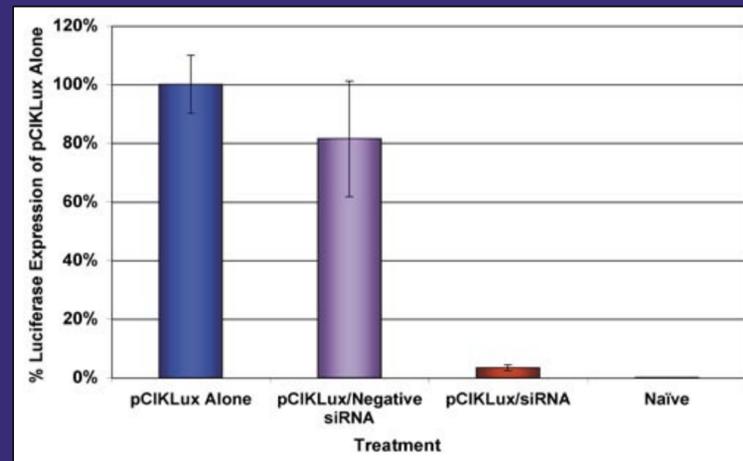
Three separate siRNA molecules were designed against unique portions each of the murine ENaC subunits. The potency of these siRNA molecules was assessed in the mouse kidney M1 cell line.

Varying levels of siRNA-mediated knockdown were observed with the differing siRNA molecules (data not shown). The most potent siRNA molecules resulted in ~85% knockdown of ENaC alpha and

siRNA in the Mouse Lung

Previous studies have observed poor siRNA-mediated knockdown of lung mRNA expression following topical administration directly to the airways. To maximise delivery, we chose an alternative hydrodynamic tail vein injection method that allows efficient siRNA delivery to many internal organs.

Efficient siRNA-mediated knockdown of expression from co-delivered plasmid DNA was observed in the lung following hydrodynamic tail vein injection (96.7±1.0% knockdown, p<0.0001).



Naked luciferase expression plasmid pCIKLux was administered to the mouse lung using hydrodynamic tail vein injection, either alone or with commercially available negative control siRNA or an anti-Lux siRNA molecule (10µg pCIKLux, 40µg siRNA in PBS; 0.1ml/g mouse weight; n=6). Luciferase activity was determined in lung homogenates 24 hours post delivery.

siRNA Knockdown of ENaC Expression in the Mouse Lung

Having established potent siRNA-mediated lung knockdown using hydrodynamic tail vein injection, we subsequently evaluated the potency of the most efficient ENaC subunit siRNA molecules.

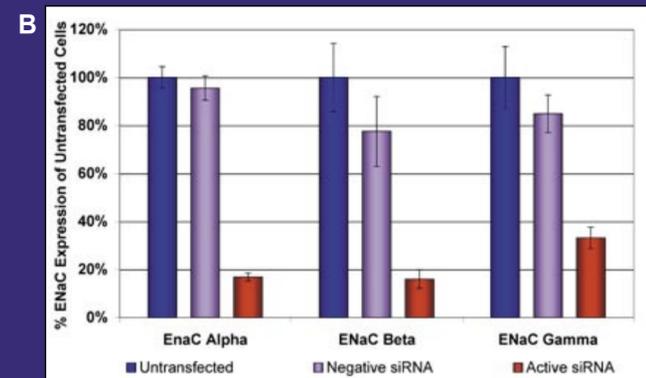
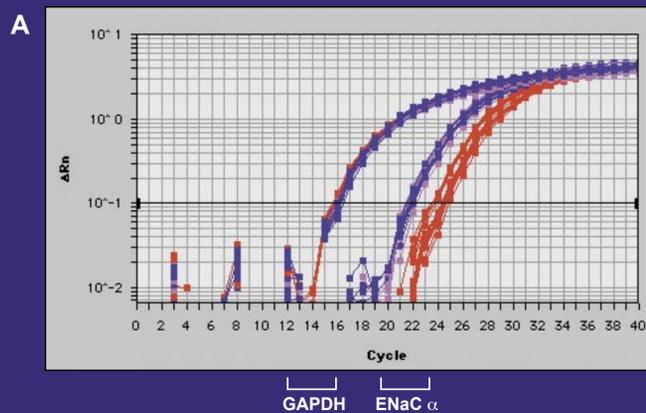
Encouragingly, hydrodynamic tail vein delivery of 40µg of the anti-ENaC alpha and anti-ENaC beta siRNA molecules resulted in ~50% and ~70% knockdown respectively (p<0.05). Disappointingly, delivery of anti-ENaC gamma siRNA was not associated with a reduction in ENaC gamma expression.

siRNA Knockdown of ENaC Expression in the Mouse Lung

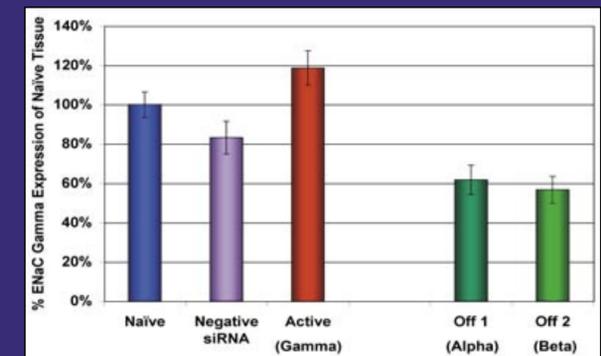
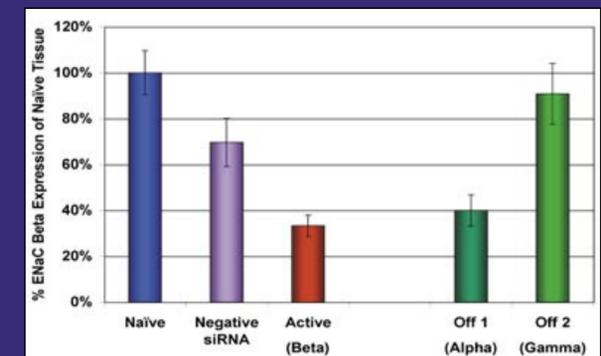
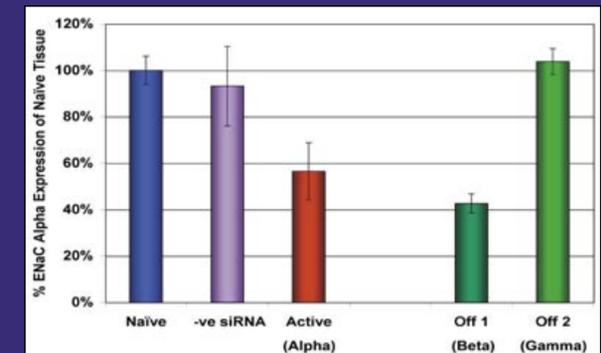
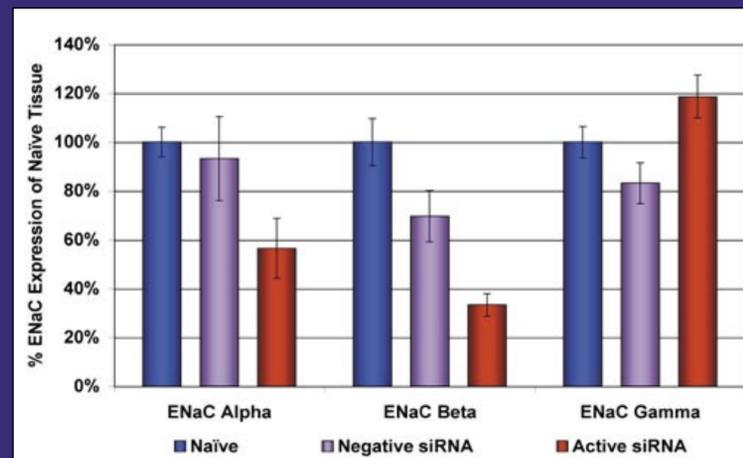
To examine 'off-target' effects of siRNA delivery, we also determined the effect of delivery of siRNA against one ENaC subunit on the expression of the other ENaC subunits.

Importantly, ENaC gamma expression was unaffected by delivery of anti-ENaC alpha or ENaC beta siRNA. Furthermore, delivery of anti-ENaC gamma siRNA had no significant effect on ENaC alpha or beta expression.

Interestingly, delivery of anti-ENaC siRNA resulted in knockdown of both ENaC alpha and beta (p<0.05). Intriguingly, this result was paralleled by the delivery of anti-ENaC beta siRNA which also resulted in the knockdown of both ENaC alpha and beta (p<0.05).



M1 cells were transfected with 20pmol of the most potent siRNA anti-ENaC molecules or a negative control siRNA using Lipofectamine 2000 (Invitrogen) and levels of ENaC subunit mRNA and GAPDH mRNA were determined using real-time TaqMan RT-PCR. (A) Real-time PCR amplification plots of GAPDH (left) or ENaC alpha (right) from representative untreated cells (blue), negative control siRNA treated cells (purple) or ENaC alpha siRNA treated cells (red). (B) ENaC subunit mRNA expression levels with most potent siRNA molecules (n=5-6). Data are represented as mean±SEM.



CONCLUSIONS

- Hydrodynamic tail vein injection can be used to assess the potency of anti-ENaC siRNA molecules *in vivo*
- Anti-ENaC subunit siRNA molecules can dramatically knockdown murine ENaC subunit expression *in vitro* and *in vivo*
- siRNA-mediated knockdown of either ENaC alpha or ENaC beta *in vivo* result in the knockdown of both ENaC alpha and beta