TARGETING αENaC WITH AN EPITHELIAL RNAi TRIGGER DELIVERY PLATFORM FOR THE TREATMENT OF CYSTIC FIBROSIS

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RATIONAL

In cystic fibrosis (CF), mutations in the CFTR chloride channel gene are associated with increased epithelial sodium channel (ENaC) activity which contributes to airway dehydration and reduced airway mucociliary transport. Hypomorphic ENaC alleles increase mucociliary transport and modify CF lung disease to milder phenotypes, but the development of inhaled small molecule inhibitors has been limited by their short duration of action and side effects resulting from renal ENaC inhibition. To enable durable, renal sparing therapeutic ENaC inhibition for CF, we have established Arrowhead’s Targeted RNAi Molecule (TRiM)™ technology to develop ARO-ENaC, an epithelial-targeted conjugate comprised of an optimized RNAi trigger against αENaC paired with an epitaxial targeting ligand (Epi) to the integral αv6 integrin.

METHODS

In vitro uptake by cultured cells was evaluated by fluorescence microscopy. Tracking conjugates were absorbed by conjugating αv6 integrin-epitaxial RNAi ligands to Cy3-labeled polycytochrome or to Cy3-labeled RNAi triggers. Integrin αv6 integrin–mediated endocytosis of Epi–RNAi conjugates was quantitated by on-cell Western receptor internalization assay (OCW–RNAI) by HT29 cells with a polyclonal antibody to αv6 receptor.

Three versions (ν1–3) of Epi–ENaC RNAi trigger conjugates were studied, employing different αv6 ligands and configurations, linker chemistries and trigger modifications.

Rats received intratracheal (IT) or oropharyngeal (OP) doses of ARO-ENaC integrin trigger conjugate aerosolized with an Aeroneb Pro vibrating mesh nebulizer. Depositional pulmonary doses were calculated using the respiratory minute volume, aerosol concentration, time, deposition fraction (estimated at 30%), and body weight.

RESULTS

αv6 ligands facilitate epithelial uptake of Cy3-labeled tracking conjugates

• HT29 cells were exposed to RNAi trigger alone or trigger conjugated to 2 different Epi ligands (A or B).
• Trigger alone does not stimulate αv6 receptor internalization by antibody bond to cell surface.
• Trigger conjugated to Epi(A) or Epi(B) stimulates αv6 receptor internalization with IC50 values of 21 nM or 359 nM.

Epi–trigger conjugates elminate expression of ENaC protein in rat airways

• Immunohistochemistry with a monoclonal antibody to αv6 receptor and confocal imaging confirm αv6–ENaC protein (red) is most abundant in airway epithelium; distal airway epithelium expresses lower levels of ENaC protein.
• In airways, αv6–ENaC protein is localized to cilia of bronchial epithelial cells, confirming previous observations.
• Single IT doses of Epi–ENaC RNAi trigger that produce ≥50% whole lung ENaC mRNA knockdown on Day 9 are associated with significantly reduced airway epithelial αv6–ENaC protein expression; remaining ENaC protein predominantly restricted to distal airway epithelium.

Epi platform increases trigger potency 10x and improves uniformity of whole lung αv6 ENaC mRNA knockdown

• Rats received three q.d. OP doses of αv6–ENaC RNAi trigger alone or Epi–Trigger conjegate and sacrificed 6 days after last dose.
• Epi–trigger conjugate reduces lung αv6 ENaC mRNA expression >50% with a total dose of 0.12 mg/kg. Equivalent knockdown with trigger alone requires 1.5 mg/kg.
• Targeting improves uniformity of lung knockdown at low exposures, consistent with improved delivery to airway epithelium.

Aerosol inhalation of EpL-ENaC trigger conjugates mediate durable whole lung αv6 ENaC mRNA knockdown

• At IT and aerosol exposures that reduce lung αENaC mRNA expression by 60% and 72%, respectively, no changes are observed in lung CFTR chloride channel or kidney αENaC mRNA expression.

CONCLUSIONS

• Epi–v6 integrin v6 receptor ligands improve endocytosis of RNAi triggers in cultured epithelial cells.
• Epi–v6 receptor–mediated conjugates are highly endocytosed by αv6 integrin receptor with IC50 values of 21 nM or 359 nM.
• A single IT dose of Epi–ENaC RNAi trigger conjugate reduces lung αENaC mRNA expression >50% with a total dose of 0.12 mg/kg.
• Targeting improves uniformity of lung knockdown at low exposures, consistent with improved delivery to airway epithelium.

REFERENCES


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