Obstacles to Translation
Conference

Overcoming obstacles to develop siRNA-based therapeutics for pachyonychia congenita

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Congenital skin disorders

10,000 congenital human diseases thought to be monogenic

• Caused by single error in single human gene
• ~20% lead to skin disorders
  – Netherton syndrome (recessive)
  – Darier’s disease and Hailey-Hailey disease (dominant)
  – Peeling skin syndrome (recessive)
  – Non-bullous congenital ichthyosiform erythroderma (recessive)
  – Epidermolysis bullosa (recessive and dominant forms)
  – Epidermolytic hyperkeratosis (dominant)
  – Loricrin keratoderma (dominant)
  – Various gap junction diseases
  – Pachyonychia congenita (dominant)
• Incidence ~1:100,000 to 1:1,000,000
• Total afflicted by rare monogenic skin disorders up to 1% of population
Pachyonychia congenita (PC)

- Autosomal dominant negative disorder resulting from mutant keratins
- 2 disease forms with similar but non-identical manifestations
  - Symptoms result from faulty keratin filaments
  - Dystrophic nails (up to 1 cm thick)
  - Palmoplantar keratosis
    - Blistering at pressure points
    - Leukoplaikia on tongue and buccal mucosa
- PC1 (Jadasson-Lewandowsky syndrome)
  - Caused by mutations in keratin 6a (K6a) or K16 genes
- PC2 (Jackson-Lawler syndrome)
  - Caused by mutations in K6b or K17 genes
- Main patient complaint--blisters on feet make walking painful and unbearable
- Goal--develop inhibitors that knock down k6a (soles of feet)
  - Working in partnership with PC-Project and International PC Consortium (IPCC)
  - No K6a probably okay
  - 50% knockdown likely therapeutic
  - Delivery of inhibitors major impediment
Challenges to a PC therapy

- Development of potent and specific inhibitors to block expression of mutant keratin with little or no effect on wildtype expression

- Effective delivery mechanism of inhibitors to affected cells
RNAi
RNA interference (RNAi)

- Dicer
- siRNA
- shRNA
- RISC
Transfected cells stained with DAPI and visualized by fluorescence microscopy

K6a-wt/YFP

K6a-N171K mut/YFP
Screening for effective N171K siRNA inhibitors

K6A WT    GAACAGATCAAGACCCTCAACAACAAGTTTGCTCCTTTC
K6A N171K GAACAGATCAAGACCCTCAAAACAAAGTTTGCTCCTTTC

Inhibitors:
K6a_513.1  ACAGAUCAAGACCCUCAAAUU
K6a_513.2  CAGAUCAAGACCCUCAAAAUU
K6a_513.3  AGAUCAAGACCCUCAAAAUU
K6a_513.4  GAUCAAGACCCUCAAAACUU
K6a_513.5  AUCAAGACCCUCAAAACAUU
K6a_513.6  UCAGACCCUCAAAACAAUU
K6a_513.7  CAAGACCCUCAAAACAAAGUU
K6a_513.8  AAAGACCCUCAAAACAAAGUUU
K6a_513.9  AAAGACCCUCAAAACAAAGUUUU
K6a_513.10 ACACCCUCAAAACAAAGUUUU
K6a_513.11 ACACCCUCAAAACAAAGUUUUU
K6a_513.12 ACACCCUCAAAACAAAGUUUGU
K6a_513.13 ACACCCUCAAAACAAAGUUUGCU
K6a_513.14 ACACCCUCAAAACAAAGUUUGCUU
K6a_513.15 ACACCCUCAAAACAAAGUUUGCUUU
K6a_513.16 ACACCCUCAAAACAAAGUUUGCUUUU
K6a_513.17 ACACCCUCAAAACAAAGUUUGCUUUU
K6a_513.18 ACACCCUCAAAACAAAGUUUGCUUUU
K6a_513.19 ACACCCUCAAAACAAAGUUUGCUUUU
Transfected cells stained with DAPI and visualized by fluorescence microscopy

K6a-wt/YFP

K6a-N171K mut/YFP
Tissue culture model of dominant negative genetic disorder
K6a wt + K6a N171K expression plasmids + indicated siRNA

Control siRNA  K6a N171K siRNA #1  K6a N171K siRNA #2

Percentage of cells containing:

Aggregates  72  4  8
Mixture      12  11 13
Filaments   16  85 79
Delivery
Delivery
Delivery
Delivery
β-actin → fLuc → 2a → eGFP → siRNA inhibitor

L2G reporter
Reporter gene expression in mouse footpad
Transgenic eGFP (L2G) mouse model

Fluorescence (Green Pseudocolor)

Cao et al., Transplantation, 2005
SiRNA-mediated inhibition of eGFP expression in transgenic mouse expressing eGFP

Day 0
Day 3
Day 6
Day 14

Top paw--eGFP siRNA
Bottom paw--NCS4 siRNA
Challenges to Translation for PC therapeutic

- Clear understanding of what will be required by FDA for IND approval
  - Lack of a good PC animal model
  - Tissue culture efficacy ≠ mice ≠ pigs ≠ humans
  - Will each siRNA will be treated as a new drug entity by the FDA (major obstacle for rare diseases such as PC) or will siRNA be treated as a class having similar toxicities
  - Sufficient patients with specific PC mutations to give meaningful results in clinical trials

- Intellectual property
  - Uncertainty over ability to operate in RNAi space and availability of appropriate licenses

- Supply of reasonably-priced synthetic siRNAs of appropriate quality

- Delivery
  - Efficient “patient friendly” delivery mechanism of nucleic acids to keratinocytes

- Limited resources
  - Partnerships with groups that see beyond the ultra-rare nature of PC and its value as a prototype for dominant negative (and other) skin disorders
  - More $s mean faster progress
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