Gene Therapy

Current Methods and Research for Cystic Fibrosis

Alexis Wallen
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What is Cystic Fibrosis?

- **Cause:** Mutation in cystic fibrosis transmembrane conductance regulator gene (CFTR) gene on Chromosome 7
- **Affects** c-AMP-regulated chloride channel in apical membrane of epithelial cells
- **Most common lethal inherited disease in whites**
Effects

- Chronic Pulmonary disease
- Progressive decline in pulmonary function
- Obstructive azoospermia in males
- Exocrine pancreatic insufficiency
- Patients usually die from lung damage or infection
Genotype

- Mutation in CFTR gene
- Usually Delta F508 mutation (70% of cases)
- Over 600 mutations documented
- Different mutations have slightly different problems
- Variation of expressivity
Delta F 508 Mutation

- Mutation in 70% of cystic fibrosis patients
- Class II biosynthetic trafficking defect
- Mutant protein degraded before it reaches cell membrane
- “Subtle defects in pulmonary function”
Gene Therapy for CF

- General Principles and Methodology
- Vectors
- Delivery
- Current Status of Research
- Challenges
General Principles

- The goal of gene therapy is to cure disease by altering the genome to include or exclude a desired set of genes.
Methodology

- Insert DNA coding for correct protein into selected vector
- Deliver vector to target site
- Vector integrates DNA into the host cell
- Desired protein product is produced by the host cell
**Terminology**

- **Recombinant**: two or more genes linked due to a crossover event
- **Transfection**: the transfer of DNA to a eukaryotic cell
- **Transduction**: the transfer of nonviral DNA by a virus to a cell
- **Vector**
What is a vector?

- Cannot simply deliver raw DNA to cells -- would be degraded, or not make it into cells
- Vector is “a DNA molecule that can carry inserted DNA and be perpetuated in a host cell”
Desired traits in CF Vector

- Low/no immune system response
- Able to integrate into non-dividing cells
- Able to integrate into epithelial cells
- Remains in body for long periods of time
- Deliver large DNA sequences
- Easy/cheap to produce in large quantities
Vectors for CF Gene Therapy

• Adenoviruses
• Adeno-associated viruses
• Liposomes
• HIV and Ebola
Adenovirus Vector

- Infect non-dividing human cells
- E1 region of cell needed for replication
- No integration into chromosome
- Can accommodate 36kb of double-stranded DNA
- In gene therapy, will replace viral replication genes with CFTR gene
Making an Adenovirus Vector

- Insert TG plasmid into adenovirus genome map units 0-17
- Introduce above and linear adenovirus DNA (map units 9-100) into host cell containing adenovirus E1 gene
Making an Adenovirus Vector

- Recombination
- Recombinant gene released during cell lysis
- No E1 for virus to replicate
- Adenovirus infects target cell
- CFTR gene expressed
Adenovirus for Cystic Fibrosis

- Vector stable in aerosol delivery
- Few cells transduced with CFTR
- Does not remain in body for long period of time
- Immune response both to vector and to transformed cells
Adeno-Associated Virus (AAV)

- Not pathogenic
- Single-stranded human DNA virus
- Integrates onto chromosome 19
- Needs proteins from helper virus (such as adenovirus)
- Host cell polymerases convert adeno-associated virus genome into double-stranded DNA which is then transcribed
Making an AAV

1. Infect a cell with helper adenovirus
2. Cotransfect the cell with two plasmids, one containing CFTR and one containing genes for replication (rep) and for capsid formation (cap)
Making an AAV

- Cell lysis releases recombinant AAV and ADV particles
- Separate AAV from ADV by centrifugation
- Deactivate any remaining ADV with heat
- Deliver recombinant AAV to patient
Adeno-Associated Virus

- Lower efficiency than adenovirus
- Limited capacity for large DNA sequences
- Phase I clinical trials:
  - No inflammatory response
  - Vector persisted 70 days
Liposomes

- Cationic-lipid-mediated gene transfer
- Can deliver large DNA sequences
- Easy to produce in large quantities
Liposomess

- No toxic effects yet observed
- Some flu-like symptoms, resolved within four days
- Significant increase in chloride channel function, but no sodium or pulmonary difference yet observed
- May reduce bacterial adherence to respiratory epithelial cells
- Transient effect
HIV/Ebola Vectors

- Most recently tried vector
- HIV persists in the body
- Ebola attaches well to lung cells
- Vector does not contain parts of the HIV and Ebola genomes needed to cause disease
- HIV genes inside of Ebola viral envelope
HIV/Ebola Vectors

• Controversy!
  – “I wouldn’t want that thing put into me.”
    – Robert Gallo, Director of Institute for Human Virology and co-discoverer of HIV
  – “It’s not even HIV anymore; it’s just pieces. And Ebola sounds horrible, but this has nothing to do with the Ebola virus that knocks out all your defense mechanisms and kills you. Those genes are gone.”
    – W. French Anderson, USC Researcher
Vector Delivery

• It is important that the vector actually have a chance to be in physical contact with the host cell.

• The best way to deliver a drug to the lungs is through inhalation.

• One of the reasons why cystic fibrosis has been popular to study is that drug delivery to the lungs is so simple!
Current Research

Includes:

• Work on HIV/Ebola vector
• Improvements to Liposome vector by adding integrin-binding motif
• Binding of adeno-associated virus to epithelial cells
References


• Rosenstein, Beryl J, Zeitlin, Pamela L. “Cystic Fibrosis.” Seminar at Johns Hopkins University School of Medicine, January 24, 1998.