Gene Therapy

Dr. Rodney Kawahara
University of Nebraska Medical Center
Department of Pharmacology
Gene Therapy

"A set of approaches for the treatment of human disease based on the transfer of genetic material (DNA) into an individual".
Therapeutic Objectives

Somatic Therapy
- Replacement of a defective gene
- Intervention in a disease process
- Alter the pharmacokinetics of drugs
- Inhibit the expression of selected genes

Germline Therapy
- Transgenic animals
- Cloning of animals
Gene Delivery

**In vivo Therapy**

Systemic administration of a gene therapy vector directly to living organisms.

**Ex vivo Therapy**

Treatment of explanted cells with the gene therapy vector followed by transplantation of the cells into a living organism.
Ideal Vector

- Selective tissue delivery
- Selective integration
- No toxic side effects
- Non-immunogenic
- Long term expression
- Regulated expression
- Delivery of large genes
- Easy to prepare
- Low cost
<table>
<thead>
<tr>
<th></th>
<th>Retrovirus and Lentivirus</th>
<th>Adenovirus</th>
<th>Adeno-associated virus</th>
<th>Herpes Simplex virus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genome</strong></td>
<td>s RNA</td>
<td>d DNA</td>
<td>s DNA</td>
<td>d DNA</td>
</tr>
<tr>
<td><strong>Genome size</strong></td>
<td>10 kb</td>
<td>35 kb</td>
<td>5 kb</td>
<td>152 kb</td>
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<tr>
<td><strong>Available size</strong></td>
<td>7.4 kb</td>
<td>30 kb</td>
<td>4.7 kb</td>
<td>50 kb</td>
</tr>
<tr>
<td></td>
<td>Retrovirus</td>
<td>Lentivirus</td>
<td>Adenovirus</td>
<td>Adeno-associated virus</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Integration</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>low</td>
</tr>
<tr>
<td>Cell proliferation</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Immune response</td>
<td>low</td>
<td>low</td>
<td>high</td>
<td>low</td>
</tr>
<tr>
<td>Virus production</td>
<td>low</td>
<td>low</td>
<td>high</td>
<td>low</td>
</tr>
</tbody>
</table>
Non-viral DNA delivery systems

- Uncomplexed DNA
- Liposomes
- DNA-protein conjugates
- Electroporation
- Gene Gun
Uncomplexed DNA

- Bacterium
- Circular plasmids (several thousand base pairs each)
- Main circular chromosome (4 million base pairs)
- Antibiotic-resistance gene
- Mobile plasmid
- Genes necessary for DNA transfer
Liposomes

<table>
<thead>
<tr>
<th>MAb</th>
<th>Receptor</th>
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<tbody>
<tr>
<td>83-14</td>
<td>human IR</td>
</tr>
<tr>
<td>528</td>
<td>human EGFR</td>
</tr>
<tr>
<td>OX26</td>
<td>rat TfR</td>
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</table>
Liposome

- Easy to prepare
- Low immune response
- No DNA size limitations

- Low targeting to specific cell types
- Moderate gene delivery
Lentivirus

Immunoliposome
DNA-Protein Conjugates

- Created by changes in salt concentration
- Transferrin/DNA polyplexes
- Gene delivery to liver and lung
- DNA remains episomal and does not integrate
Electroporation

- Ex vivo therapy
- Electrical current is used to create membrane pores
- Both transient and stable DNA transfer is possible
Gene Gun
<table>
<thead>
<tr>
<th></th>
<th>Naked DNA</th>
<th>Liposome</th>
<th>DNA Protein</th>
<th>Electro-poration</th>
<th>Gene Gun</th>
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</thead>
<tbody>
<tr>
<td><strong>Size Limit</strong></td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td><strong>DNA Delivery</strong></td>
<td>low</td>
<td>variable</td>
<td>low</td>
<td>low</td>
<td>low</td>
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<tr>
<td><strong>Expression</strong></td>
<td>transient</td>
<td>transient</td>
<td>transient</td>
<td>transient</td>
<td>transient</td>
</tr>
<tr>
<td><strong>Immune response</strong></td>
<td>low</td>
<td>low</td>
<td>variable</td>
<td>N/A</td>
<td>variable</td>
</tr>
<tr>
<td><strong>Tissue</strong></td>
<td>muscle</td>
<td>lung</td>
<td>potential</td>
<td>ex vivo</td>
<td>skin liver</td>
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<tr>
<td></td>
<td></td>
<td>liver</td>
<td>specific</td>
<td>cultured cells</td>
<td></td>
</tr>
</tbody>
</table>

- Naked DNA: Protein poration
- Liposome: Gene gun
- DNA: Low delivery, variable expression, low immune response
- Electro-poration: Low delivery, transient expression, variable immune response
- Gene Gun: Low delivery, transient expression, variable immune response
Methods used to alter gene expression

- Antisense oligonucleotides
- Ribozymes
- siRNA
Antisense - Backbones

- Phosphorothioate oligonucleotide
- Peptide nucleic acid oligonucleotide
- Methylene methyl imino oligonucleotide
5' Cap | Leader | Translational Start Site | Amino Acid Coding Region

I

7mGpppACUGAGAAGGACCUGUGCUUGCUCGCUGACAAAGACC

Translational Start Target

3'-TACCACGACAGAGGGCGACTGTTTCT-5'

RNase H Cleavage
Formivirsen (Vitravene)

- First antisense compound approved for marketing
- Phosphorothioate oligonucleotide
- GCG TTT GCT CTT CTT CTT CTT GCG (21 bases long)
- Local treatment of cytomegalovirus retinitis
- Inhibits CMV replication
- Complementary to the major immediate early region 2
Administration, Metabolism, Elimination

- Intravitreal Injection
- Metabolized by exonucleases
- Eliminated in the urine, feces and catabolized to carbon dioxide
Resistance and Side effects

- Effective against ganciclovir, foscarnet and cidofovir resistant CMV
- Resistant CMV reported
- Intravitreal injection does not produce systemically detectable drug
- Increased intraocular pressure
Ribozymes

- Catalytic RNA molecules that digest mRNA targets
- Hybridization to specific targets
siRNA

- Double stranded RNA
- Less than 30 bp
- Incorporated into large multiprotein complex
- Single stranded guide RNA guides the complex by binding to the target RNA
- Ribonuclease destroys the target RNA
Methods used to repair genes

- RNA-DNA hybrids
- Homologous recombination of larger DNA fragments
Wild Type  CAT  AGG  CTT  GGT  TAT
Mutant  CAT  AGG  CTA  GGT  TAT
Converted  CAT  AGG  CTT  GGT  TAT

TGC CGCGauccguauccGAACCGaauacggcccaT
T
T
T

TCGC  GCTAGGCATAGGCTTGGTTATGCCGGTT
3'  5'

RNA/DNA HYBRID
Problems with gene therapy

- DNA delivery
- Expression of the gene product
- Long term stable expression
- Adverse response to the gene product
- Immunological responses
Ornithine Transcarbamylase Deficiency

- Jesse Gelsinger
- Adenovirus, IV high dose
- Unusual and deadly immune-system response
- Multiple organ failure
Severe Combined Immunodeficiency (SCID)
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Severe Combined Immunodeficiency (SCID)

- Adenosine Deaminase (ADA)
- September 1990
- Ashanti DeSilva
- Cynthia Cutshall
Severe Combined Immunodeficiency (X-SCID)

- Common gamma chain for IL-2,4,7,9,15,21 receptors
- Murine Leukemia Virus
- Integration and disruption of the LMO-2 gene
- Two of Nine children develops ALL
Severe Combined Immunodeficiency (X-SCID)

- Additional child with leukemia (3/17)
- LMO-2 gene unaffected
- Three sites of integration
Inherited Disorders
- ADA deficiency

Monogenetic Disorders
- Cystic fibrosis
- Sickle cell anemia

Infectious Diseases
- HIV

Acquired Disorders
- Peripheral artery disease
- Rheumatoid arthritis

Cancer
- Immunotherapy

Cell Marking Protocols
- Experimental
Diseases being investigated in gene therapy clinical trials.

- Infectious disease: 13%
- Other: 2%
- Gene marking: 7%
- Monogenic disorders: 9%
- Cancer: 69%