Gene Therapy and Genetic Counseling

Chapter 20
What is Gene Therapy?

• Treating a disease by replacing, manipulating or supplementing a gene

• The act of changing an individual’s DNA sequence to fix a non-functional gene

• Altering a person’s genotype in the area where it is malfunctioning
Methods of Gene Therapy

• Normal gene is inserted at random into the genome so that functioning protein is made
• Homologous recombination used to swap the normal gene for the abnormal gene
• Abnormal gene is repaired through site directed mutagenesis (“mutating” sequence to it’s normal form)
• Changing the regulation of the gene
  – Changes the amt of protein
Random Insertion

Put a functioning gene into a DNA vector

• Administer vector into human cells:
  – Usually administered through a defunct virus
  – Viruses have evolved efficient ways of inserting DNA into genome
  – Virus with vector administrated to specific cell types depending on disease

• Functional gene is randomly inserted into human’s genome and then translated to functional protein
DNA Vector

Gene

Vector

Defunct Virus
Virus Infection

Cell Type that needs protein → Virus used to insert DNA vector into specific cell → New DNA integrates into cell’s genome: expressing protein
Homologous Recombination

Replace the broken gene with a functional version

Mutation Occurs

Crossing over based on homology of regions

Functional protein:
Site Directed Mutagenesis

Actually change the broken gene’s sequence to regain it’s natural function

• Have to know exactly what the mutation is
• Then add mutagens that will introduce the “mutation” you want to change the gene back to it’s natural sequence again
• Some mutagens introduce transversions, some add or remove specific bases, etc
• What would be a problem with this?
Mechanisms

(a) Recombinant plasmid DNA
- Gene
- restriction endonucleases
- Oligonucleotide with sequence change
- DNA ligase
- DNA polymerase, dNTPs, DNA ligase
- transformation of E. coli cells; repair of DNA

(b) Plasmid contains gene with desired base-pair change.
In E. coli cells, about half of plasmids will have gene with desired base-pair change.
Changing Regulation

Change the promoter or regulatory sequences so that the gene’s expression is increased or decreased

- If disease is caused by misexpression or underexpression of gene
- Use either homologous recombination or site directed mutagenesis to change the regulatory regions of gene
Pros vs. Cons

• Which method do you think would be the best?
• What are the pros and cons of each:
  – Random insertion
  – Homologous recombination
  – Site directed mutagenesis
  – Changing regulation
• Depends on type of mutation/disorder
The Best Chance of Working

• Know exactly what is causing disorder
  – What gene
  – What mutation
• Know the biology of what is occurring in disease vs. normal states
• Single gene
• High penetrance
• Genetically homogeneous
Proteins vs. Genes

- Text discusses some “gene therapies” that are not actually affecting the genes.
- Administering proteins or enzymes is not gene therapy.
- Treating the symptoms of the disorder is not gene therapy.
- It’s only gene therapy when it’s actually changing/adding the DNA sequence.
Examples

• ADA – Adenosine Deaminase Deficiency
  – First administered missing enzyme
  – Then began giving white blood cells that had functional gene inserted into them

• OTC – Ornithine Transcarbamylase Def.
  – Functional OTC gene administered through defunct virus

• Canavan Disease
  – Introduce raw DNA in liposome through hole in skull
Invasiveness of Therapy

• **Ex vivo gene therapy:**
  – Remove cells, correct DNA outside body, and then administer corrected cells

• **In situ gene therapy:**
  – Healthy gene and vector are administered to localized cell type that is affected

• **In vivo gene therapy:**
  – Vector is administered directly to blood stream or fetal cells
  – May spread to entire body
Germline vs. Somatic

• Germline gene therapy:
  – Changing the DNA in the germline cells so that offspring will be completely normal
  – Entire body of offspring
  – Heritable change
  – Not done in humans

• Somatic gene therapy:
  – Treating specific cell types that are affected
  – This is what we can do now
Questions and Concerns

• What protein is missing/non functional?
• What gene encodes that protein?
• What is mutation in that gene?
• Is there genetic heterogeneity?
  – More than one mutation, or gene involved
• What cells/tissue is affected?
• Will gene therapy affect other cells and how?
Questions and Concerns

• What DNA sequence surrounds mutation?
• How much DNA must be corrected?
• How many cells must be corrected?
  – All or only some enough?
• How long will correction persist?
  – Some cells die quickly, and therefore treatment would have to be repeated
• How will immune system respond to treatment?
Sites of Therapy

Brain
- growth factors
- Alzheimer disease
- Huntington disease
- neurotransmitter imbalances
- spinal cord injuries
- leishma genes
- glioma
- tyrosine hydroxylase
- Parkinson disease

Skin
- HLA-B7 melanoma

Blood
- beta globin sickle cell disease

Endothelium (blood vessel lining)
- clotting factors
- hemophilies
- insulin diabetes mellitus
- growth hormones pituitary dwarfism

Transplant
- Bone marrow
  - glucocerebrosidase
  - Gaucher disease
  - adenosine deaminase (ADA) severe combined immune deficiency

Liver
- CFTR cystic fibrosis
- alpha-1-antitrypsin hereditary emphysema
- LDL receptor familial hypercholesterolemia
- Implanted retroviruses

Muscle
- dystrophin

Aerosol
- Implanted retroviruses

Injection
- Naked DNA

Transplant
- AV = adenovirus
- AAV = adeno-associated virus

Myoblast transfer
- liposomes
Gene Delivery

Methods for inserting foreign DNA into cells:

• Simply forcing raw DNA into nucleus:
  – Microinjection, electroporation

• Enclosing DNA in a vehicle to cross plasma membrane:
  – Liposomes (vesicles of fat surrounding DNA)
  – Liposomes with integrin protein that binds target cell type
  – Defunct viruses
Viruses

- Remove parts of virus that cause symptoms or disease
- Also remove parts of virus that trigger immune reaction
- Add corrected gene to viral DNA
- Administered this virus to specific cells that need functional protein
- Choose “best” virus for the job
Viruses

Different viruses can do different things:

• One virus may be very efficient, but can only carry very small piece of DNA
• Another virus may carry large string of DNA but send it to too many cell types
• Another virus may not infect enough of the appropriate cell type to produce effect
• Some viruses only infect dividing cells
## Different Viruses

<table>
<thead>
<tr>
<th>Vector</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adeno-associated</td>
<td>Integrates into specific chromosomal site</td>
</tr>
<tr>
<td>Adenovirus (AV)</td>
<td>Long-term expression</td>
</tr>
<tr>
<td></td>
<td>Nontoxic</td>
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<tr>
<td></td>
<td>Infects dividing and nondividing cells</td>
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<tr>
<td></td>
<td>Carries small genes</td>
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<tr>
<td>Herpes</td>
<td>Large virus, carries large genes</td>
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<tr>
<td></td>
<td>Transient expression</td>
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<tr>
<td></td>
<td>Evokes immune response</td>
</tr>
<tr>
<td></td>
<td>Infects dividing and nondividing cells, particularly in respiratory system</td>
</tr>
<tr>
<td>Retrovirus</td>
<td>Long-term expression</td>
</tr>
<tr>
<td></td>
<td>Infects neuroglia</td>
</tr>
<tr>
<td></td>
<td>Stable but imprecise integration</td>
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<tr>
<td></td>
<td>Long-term expression</td>
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<tr>
<td></td>
<td>Most types infect only dividing cells</td>
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<tr>
<td></td>
<td>Nontoxic</td>
</tr>
<tr>
<td></td>
<td>Most established in clinical experience</td>
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Summary of Gene Therapy

• Define gene therapy correctly
• Know different mechanisms of administering gene therapy
• Know differences:
  – Invasiveness of treatment
  – Germline vs. somatic
  – Methods of gene delivery
• Know what questions need to be answered for gene therapy to work
Genetic Counseling

Public health professionals who help communicate clinical genetics to patients

• May be:
  – Nurses, PhD’s, social workers, physicians

• Draw up pedigrees, explain outcomes of genetic testing and calculate likelihoods

• Counsel families on how to understand and proceed based on genetics
How to become a Genetic Counselor

• Have a graduate level degree:
  – Masters, RN, MD, PhD
• Earn a degree from a Genetic Counseling program
• Pass a certification to become board certified to practice
• Willingness to counsel and support
Who are Genetic Counselors?

- Must be certified in most states

In USA mostly:
- Women (95.3%)
- White (97%)
- 25-35 years old (58%)
- Have Masters degree in Genetic Counseling (66%)

According to NSGC 2004 Survey
Where do they work?

Can work in any of the following situations:

• Medical Centers
• Hospitals
• Private Clinics/Practices
• Infertility/Reproduction Centers
• Research/pharmaceuticals companies
• Government programs
• 41% work in University Hospitals

According to NSGC 2004 Survey
Genetic counselors have two major roles:

1. Understand and be able to use genetics
   - Draw up pedigrees, calculate likelihoods
   - Analyze genetic testing results

2. Counsel patients and family members on what genetics means to them
   - Counsel couples on genetic disorders
   - Refer families to support
   - Balance/explain possible risks
Recommend Genetic Tests

Genetic counselors often recommend which genetic screening tests are useful:

• **Prenatal**
  – Family with inherited disorder
  – Certain risk factors (maternal age, exposure)

• **Newborn**
  – Some necessary, some optional

• **Adult**
  – To explain symptoms or for inherited risk
Prospective Parents

Genetic Counselors often counsel prospective parents

• Analysis of population/empiric risk
• Analysis of family history/background
  – Draw pedigree for couple
• Explanation of genetic tests and risks
• Interpretation of results of tests
• Counseling if results are worrisome
Inherited Disease

Genetic Counselors also counsel families with known inherited disorders

• Draw up pedigree or analyze pedigree already known

• Counsel patients and family members on potential treatments

• Counsel families on subsequent pregnancies and risks of having affected children
Summary of Genetic Counselors

• Know training necessary
• Know what they do:
  – Provide information on inheritance, disease risks, available tests, treatments and options
• Differences when dealing with prospective parents vs. families with a particular genetic disorder
Let's Talk about Ethics…

• Get into pairs

• Discuss what you would do as the genetic counselor in each of the situations

• Make notes of your discussion

• Then we’ll all share our opinions with the rest of the class
What would you do?

1. Couple has suffered many miscarriages, finally has a child with multiple severe problems. Genetic testing finally reveals father has chromosomal translocation. He has 5 siblings, many of which could have the same translocation. He refuses to tell his siblings of his test results.

What do you do? What are your medical obligations? How do you feel about them?
What would you do?

2. A couple has the same autosomal recessive form of blindness. They want to have children even though they know their children will all be blind.

Do you recommend adoption or IVF with donor sperm and zygote?

What could be the problems with recommending these options?
What would you do?

3. A couple has a child with PKU. The special diet costs $8,000 per year. The woman becomes pregnant again but this time her insurance refuses to pay for the $1,200 prenatal test that will diagnose PKU. They also refuse to pay to feed the second child.

The couple asks you whether they should terminate the pregnancy. What is your advice to them?
What would you do?

4. A subway driver discovers that he has familial hypercholesterolemia. Although he sometimes experiences chest pains, he has never had a heart attack. He knows he may suddenly have a heart attack while working, but yet he needs a job to pay for his NYC rent and for his family.

What do you recommend he do? Should he tell his employers?

Assume you ride the subway everyday.
Next Class:

• Read Chapter 21

• Homework – Chapter 20 Problems;
  – Review: 3, 5, 8, 9
  – Applied: 3, 5, 11, 12, 13