Gene Therapy

Dr. W. French Anderson with four-year-old Ashanthi De Silva at U.S. National Institutes of Health
Gene Therapy not a fantasy now.....

• In 1990, a 4 year old girl named Ashanthi De Silva was the first patient to receive gene therapy for SCID (severe combined immunodeficiency).

• She became a healthy adult with an immune system that was able to fight off most infections.
What is the outcome of gene therapy?

- Gene therapy -
  - replaces a mutated gene with a healthy one
  - deactivates a gene that isn’t functioning properly
  - introduces a new gene in the body to help fight the disease
  - Enhances the effect of a normally functioning gene.
  - Activates the gene that was shut down during fetal life.
## Types of Gene Therapy

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<th>Somatic Cell Gene Therapy</th>
<th>Germ Line Gene Therapy</th>
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<tr>
<td>Therapeutic genes transferred into the <strong>somatic cells</strong>.</td>
<td>Therapeutic genes transferred into the <strong>germ cells</strong>.</td>
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<td>Eg. Introduction of genes into bone marrow cells, blood cells, skin cells etc.</td>
<td>Eg. Genes introduced into eggs and sperms.</td>
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<td><strong>Will not be inherited</strong> later generations.</td>
<td><strong>It is heritable</strong> and passed on to later generations.</td>
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<td>At present all researches directed to correct genetic defects in somatic cells.</td>
<td>For safety, ethical and technical reasons, it is not being attempted at present.</td>
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Germ line Gene Therapy

- normal version of gene is inserted into germ cells
  - those germ cells will divide normal versions of the gene
  - any zygote produced as a result of this germ cell will have a correct version of the defective gene and will continue passing it on to their offspring
Somatic cell gene therapy

IN VIVO

EX VIVO
IN VIVO GENE THERAPY

- **Direct delivery** of therapeutic gene into target cell into patients body.

- Carried out by viral or non viral vector systems.

- It can be the only possible option in patients where individual cells cannot be cultured in vitro in sufficient numbers (e.g. brain cells).

- In vivo gene transfer is necessary when cultured cells cannot be re-implanted in patients effectively.
1. Cells are removed from patient.

2. In the laboratory, a virus is altered so that it cannot reproduce.

3. A gene is inserted into the virus.

4. The altered virus is mixed with cells from the patient.

5. The cells from the patient become genetically altered.

6. The altered cells are injected into the patient.

7. The genetically altered cells produce the desired protein or hormone.
Somatic Cell Gene Therapy

- single defective cell taken out of an individual’s body
- functional version of gene introduced into cell in a laboratory
  - cells reproduce
  - copies of cells with a corrected version of the gene is injected back into the patient
- the good gene ends with the patient and is not inherited by their offspring
How to introduce the genes?

- There are three ways-

- **Ex vivo strategy** - Where the patients cells are cultured in the laboratory, the new genes are infused into the cells and modified genes are administered back to the patient.

- **In situ strategy** - Where the carrier of the gene is injected to the patient either intravenously or directly to the tissues.

- **In vivo strategy** - Where the vector is administered directly to the cell.
Summary of the procedure

• Isolate the healthy gene along with its regulatory sequence to control its expression.
• Incorporate this gene on to a vector or carrier as an expression cassette.
• Deliver the vector to the target cells.
What are Vectors and why are they needed?

• Different carrier systems are used for gene delivery:
  1) Viral systems
  2) Non viral systems

Vectors are needed since the genetic material has to be transferred across the cell membrane and preferably into the cell nucleus.
Viral vectors

- Retroviruses
- Adeno viruses
- Adeno associated viruses
- Herpes simplex viruses
The retroviruses are modified to carry genes. The *gag, pol, env* genes are deleted rendering them incapable of replication inside the host cell. Viruses are then introduced into a culture containing the helper viruses. The helper virus is an engineered virus which is deficient in Ψ segment, but contains all other genes for replication. That means it has the genes to produce viral particles but lacks the genes required for packing.
Retroviruses as vectors

The replication deficient but infective retro virus vector carrying the human gene now comes out of the cultured cells. These are introduced into the patient. The virus enters the cell via specific receptors. In the cytoplasm of the human cells, the reverse transcriptase carried by the vector converts the RNA to DNA, which is then integrated into the host DNA. The normal human gene can now be expressed. The integrated DNA becomes a permanent part of the chromosome.

- The three genes of viral genome are removed and the desired segment of DNA, also called as cassette DNA, is inserted. Now a mutant virus is formed having our choice of gene.
Viral RNA

LTR  ψ  gag  pol  Env  LTR

Reverse transcriptase

DNA

LTR  ψ  gag  pol  Env  LTR

Gene of interest

Recombinant DNA technology

LTR  ψ  gene of interest  LTR

Helper Virus RNA

LTR  ψ  gag  pol  Env  LTR

Reverse transcriptase

DNA

LTR  ψ  gag  pol  Env  LTR

Recombinant DNA technology

LTR  gag  pol  Env  LTR

Culture cell membrane

Source of these enzyme is helper virus, enzyme are biocatalyst which is not finished. So both virus can utilize it.

Integrate

Polymerase

Insertion of cassette gene into the cell DNA.

Formation of many daughter viruses

Can’t form daughter virus because of lack of ψ segment, which is essential for integration of viral components.

Integrate

Polymerase

Insertion of cassette gene into the cell DNA.

Viruses with enzymes polymerase, integrate, core protein & mutant RNA are produced which is injected in host cell. Remember this virus don’t have gene gag, pol, & env. Instead contains ψ gene.
Advantages and Disadvantages?

Advantages- The virus is replication deficient, so it's safe and is suitable for the treatment of a variety of diseases.

Disadvantages-
1) Random insertion can disrupt normal genes.
2) Retroviruses use rapidly dividing cells as targets. The non dividing cells can not be used.
Adeno viruses

- These are DNA viruses. These do not produce serious illness so are used for gene therapy. The genes of the virus are removed so they lose the ability to divide. The human genes are inserted and the vector is transfected in the culture containing the sequences for replication. The virus thus replicates in the cell culture. The packed viruses are then introduced in to the patient. It is not integrated but remains as Epichromosomal.
Gene therapy using an adenovirus vector
• **Adeno associated virus** - It is also DNA virus. It has no known pathogenic effect and has wide tissue affinity. It integrates at a specific site.

• **Herpes simplex virus** - This is a disabled single copy virus and has **defective glycoprotein**. When propagated in the complementary cells, viral particles are generated. Since they can replicate only once so there is no risk of a disease.
Herpes Simplex Viruses

• Double stranded DNA viruses that infect neurons
• Ex. Herpes simplex virus type 1

http://www.ucmp.berkeley.edu/alllife/virus.html
What are non viral systems?

1) Spontaneous uptake by endocytosis
2) Plasmid liposome complex
3) Uncovered plasmids
4) Gene gun methods
5) Electroporation
6) Microinjections.
Non viral approach - liposomes

Liposome DNA carrying the gene of interest Target cell

The liposome is degraded within the endosome and the DNA is released into the cytosol.
The DNA is imported into the cell nucleus.

(a) Nonviral approach

By recombination, the DNA carrying the gene of interest is integrated into a chromosome of the target cell.
Non-viral Options

• Direct introduction of therapeutic DNA
  – But only with certain tissue
  – Requires a lot of DNA
• Creation of artificial lipid sphere with aqueous core, liposome
  – Carries therapeutic DNA through membrane
• Chemically linking DNA to molecule that will bind to special cell receptors
  – DNA is engulfed by cell membrane
  – Less effective 😞
• Trying to introduce a 47th chromosome
  – Exist alongside the 46 others
  – Could carry a lot of information
  – But how to get the big molecule through membranes?
Current Status

• FDA hasn’t approved any human gene therapy product for sale

Reasons:

• In 1999, 18-year-old Jesse Gelsinger died from multiple organ failure 4 days after treatment for ornithine transcarboxylase deficiency.
  – Death was triggered by severe immune response to adenovirus carrier

• January 2003, halt to using retrovirus vectors in blood stem cells because children developed leukemia-like condition after successful treatment for X-linked severe combined immunodeficiency disease
Problems with Gene Therapy

• **Short Lived**
  – Hard to rapidly integrate therapeutic DNA into genome and rapidly dividing nature of cells prevent gene therapy from long time
  – Would have to have multiple rounds of therapy

• **Immune Response**
  – New things introduced leads to immune response
  – Increased response when a repeat offender enters
• Viral Vectors
  – patient could have toxic, immune, inflammatory response
  – also may cause disease once inside
• Multigene Disorders
  – Heart disease, high blood pressure, Alzheimer’s, arthritis and diabetes are hard to treat because you need to introduce more than one gene. May induce a tumor if integrated in a tumor suppressor gene because of insertional mutagenesis
Accomplishments of Gene Therapy

• **Severe combined Immuno deficiency (SCID)** - It is caused by the deficiency of adenosine deaminase enzyme. The first trial of gene therapy was done on this disease. Follow up studies show the presence of normal immune functions in recipients compatible with life.
Successful One Year Gene Therapy Trial For Parkinson's Disease

• Neurologix a biotech company announced that they have successfully completed its landmark Phase I trial of gene therapy for Parkinson's Disease.

• This was a 12 patient study with four patients in each of three dose escalating cohorts. All procedures were performed under local anesthesia and all 12 patients were discharged from the hospital within 48 hours of the procedure, and followed for 12 months. Primary outcomes of the study design, safety and tolerability, were successfully met. There were no adverse events reported relating to the treatment.
The gene transfer procedure utilized the AAV (adeno-associated virus) vector, a virus that has been used safely in a variety of clinical gene therapy trials, and the vehicle that will be used in all of the company's first generation products, including epilepsy and Huntington's disease. In its Parkinson's disease trial, Neurologix used its gene transfer technology.
Recent Developments

• Genes get into brain using liposomes coated in polymer call polyethylene glycol
  – potential for treating Parkinson’s disease
• RNA interference or gene silencing to treat Huntington’s
  – siRNAs used to degrade RNA of particular sequence
  – abnormal protein won’t be produced
• Create tiny liposomes that can carry therapeutic DNA through pores of nuclear membrane
• Sickle cell successfully treated in mice
Accomplishments of Gene Therapy

• Restenosis – 13 patients were treated by DNA carrying genes for angiogenesis. All were improved.
• Breast cancer, prostate cancer, lung cancer, brain cancers and ovarian cancers treated.
• Activation of Hb F gene in patients of Thalassemia and sickle cell diseases.
• Trials to enhance the genes of intelligence, height and athleticism.
• Trials to treat the individuals with genetic predisposition to conditions such as asthma, alcoholism, Alzheimer's disease. Schizophrenia, manic depression and Breast cancer before the onset of clinical manifestations.
Genetic disorders –
1) Duchenne Muscular dystrophy
2) Cystic fibrosis
3) Familial hypercholesterolema
4) Hemophilia
5) Haemoglobinopathies
6) Gaucher’s disease
7) Albinism
8) Phenyl ketonuria.
Acquired diseases

- Cancers
- Infectious disease - HIV
- Neurological disorders
- Cardiovascular diseases
- Rheumatoid arthritis
- Diabetes mellitus
Transgenesis

• The human genes are microinjected to farm animals and transgenic constructs have been produced.
• Factor IX protein is expressed in the milk of transgenic sheep
• Transgenic pigs produce human blood suitable for transfusion
• Transgenic mice produce human Hb with the same oxygen carrying capacity.
However...

- gene therapy is still in its early stages and is far from perfection
  - it can only be used in diseases caused by a single gene malfunction
- many diseases are caused by multiple genes
  - it can be hard to get a good gene to the specific place it needs to be
- more damage can be caused by genes being put in the wrong place
  - death can result due to infections and invasions of other viruses
Ethical Issues

- Who decides what is normal and what is a defect?
- What kind of an impact will this have on people who are currently living with these disabilities. Will this make them feel worse about themselves?
- Gene therapy is expensive so will only the rich have access to treatment? What will happen to the poor?
It is possible that gene therapy given to an adult could reverse a genetic disease. If so, would that therapy also prevent any children the person had after gene therapy from inheriting the disease?

Somatic cell treatment stays with the individual, germ cell treatment passes down the germ line (becomes immortal).
Problems

- Acute immune response to viral vectors
- Repeated treatment needed
- Genes “lost” when the cell goes through mitosis
- Viral vectors could become pathogenic
- Genes spliced at random into the genome could upset other genes
- Multigene disorders too complex to treat
Applications

• Curing genetic diseases
• Correcting cancer genes
• Inducing cancerous cells to make toxins so they kill themselves
• Blocking viral genes (e.g. HIV)
• Creating stem cells from somatic cells
gene therapy

would you say you had a dominant or recessive character?
GOOD LUCK

THANK YOU

GOOD LUCK