“GENE THERAPY”

Dr. Aditi Lal Koul
VPB
“We used to think that our fate was in our stars, but now we know that, in large measure, our fate is in our genes”

-James Watson
Gene

- Basic unit of heredity
- Are carried on a chromosome
- DNA $\rightarrow$ RNA $\rightarrow$ proteins
- Mutation in the gene causes change in the conformation & function of the protein
What is Gene Therapy?

- ‘the use of genes as medicine’

- Replacing a defective gene with a normal gene thus restoring the lost gene function (Culver, 1994)
William French Anderson

W. French Anderson
Father of Gene Therapy
by Rob Burke and Barry Epperson
Prerequisites for candidate disease

- incurable, life-threatening

- Organ, tissue and cell types affected by the disease should be identified

- The Normal Counterpart Of The Defective Gene isolated and cloned

- The balance of the risks and benefits of gene therapy for disorder must be favourable to other available therapies.

- Safety trials of procedure.

(Nicholas, 1988)
How It Works.....

ADMINISTRATION
- Introduction of vector containing gene into the body

DELIVERY
- Transfer of gene from site of administration to nucleus of target cell

EXPRESSION
- Production of therapeutic gene product in cell

Jain, 2008
Types of Gene Therapy

Somatic gene therapy

- In vivo gene therapy
- Ex vivo gene therapy

Germ line gene therapy

(Lasic et al., 1995)
Therapeutic genes are transferred into the somatic cells of a patient. Effects restricted to the individual patient only. Will treat symptoms but do not cure disease. (Lasic et al., 1995)
In Vivo Gene Therapy

- DNA liposome
- Tissue injection
- Recombinant virus
- Systemic infusion
- Biolistic gene gun
- plasmid DNA

(Wilson, 1996)
1. Removal of cells
2. Making viral replication defective
3. Corrective gene insertion into virus
4. Transfection of cell
5. Genetically altered cell
6. Injected back in body
7. Genetically altered cells in action

(Wilson, 1996)
Germ cells, i.e., sperm or eggs or embryo are modified by the introduction of functional genes.

Effect will be present on both somatic and germ cells.

Heritable and passed on to later generations.

(Lasic, 1995)
BASIC MECHANISMS OF GENE THERAPY

Replacing Absent or Defective Gene/Protein.

Gene Silencing.

Enhanced Production of Beneficial Protein.
Viral vectors

- Retroviruses
- Adenoviruses
- Adeno-associated virus
- Herpes simplex virus
- Lentivirus

Non-viral method

- Naked DNA

(Cotrim and Baum., 2008)
Vectors deliver genes to cells

Vector for efficient gene delivery

Transcription

Translation

Therapeutic protein

Therapeutic gene (Transgene)

(Rosenberg et al., 2000)
A. Retro Viral Vectors

Therapeutic Gene
Limitations of Retroviral Vectors

- Inability to infect non-dividing cells
- Insertional mutagenesis
- Low virus titres
B. Adenovirus

- Non enveloped particle
- Contains linear double stranded DNA
- Does not integrate into the host genome
Replication-deficient adenovirus vectors Rad are generated by replacing the E1a or E1b gene

RAd containing Therapeutic gene is left free in the nucleus of the host cell and are transcribed

These extra genes are not replicated when the cell is about to undergo cell division so the descendants of that cell will not have the extra gene (Krisky, 1998)
Limitations of Adenoviral vectors

- Limited duration of transgene expression after in vivo delivery
- Activates Humoral Response

(Smith et al., 1997)
NON-VIRAL VECTORS

➢ Gene Transfection using Naked DNA

➢ Direct injection into tissue / systemic injection

➢ Simple, safe & lack of specific immune response

➢ Two approaches for gene delivery-
  ✓ Physical: Electroporation, Sonoporation, Gene gun
  ✓ Chemical: lipids & polymers

(Jiao et al., 1992)
Physical Methods for gene delivery

Vein Injection
Blood Occlusion
Electroporation
Portal Injection
Artery Injection
Local Injection
Gene Gun
Ultrasound

Niidome & Huang, 2002
ELECTROPORATION

**Before Pulse**

- Cell membrane

**During E-field**

- Introduce genes/drugs
- Electric field induces a voltage across cell membrane

**After Pulse**

- Cell "heals" with gene/drug inside
Gene Gun

- Convenient – hand held
- Achieves direct gene delivery into tissues
- DNA coated with gold particles
Some other physical methods for use in enhancing gene delivery are:

- Molecular vibration
- Magnetofection
- Hydrodynamic delivery
- Laser irradiation
- Photochemical transfection etc..
By chemically linking the DNA to a molecule that will bind to special cell receptors:

- CaPO4 precipitation
- Polycationic compounds
- Oligonucleotides
- Liposomes
- Polyplexes
- Inorganic nanoparticles
- Cell penetrating peptides

(Jain, EOLSS)
Insert DNA with ligand

Complexation of DNA

Binding to the receptor

receptor

endocytosis

endosome

nucleus

Nuclear trafficking
Lipoplexes and Polyplexes

Protect DNA from degradation during transfection and entry to cell is facilitated

Lipoplexes
- Cationic lipids condense with negatively charged DNA molecules
- Cells uptake lipoplexes by endocytosis
- Fusogenic lipids - destabilize endosomal membranes
- Helper lipids - electroneutral lipids to increase transfection efficiency
- Useful in Cancer therapy, Genetic Respiratory diseases

Polyplexes
- Polyplexes are complexes of polymers with DNA
- Cationic polymers are used
- Endosome lytic agents like inactivated adenovirus are required along with polyplexes

(Radler, 1997)
# Candidates for Gene Therapy

<table>
<thead>
<tr>
<th>Disease</th>
<th>Defect</th>
<th>Incidence</th>
<th>Target Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe combined immunodeficiency (SCID)</td>
<td>Adenosine deaminase (ADA) in 25% of SCID patients</td>
<td>Rare</td>
<td>Bone-marrow cells or T lymphocytes</td>
</tr>
<tr>
<td>Hemophilia</td>
<td>Factor VII deficiency</td>
<td>1:10,000 males</td>
<td>Liver, muscle, fibroblasts or bone marrow cells</td>
</tr>
<tr>
<td>A</td>
<td>Factor IX deficiency</td>
<td>1:30,000 males</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Familial hypercholesterolemia</td>
<td>Deficiency of low-density lipoprotein (LDL) receptor</td>
<td>1:1 million</td>
<td>Liver</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>Faulty transport of salt in lung epithelium</td>
<td>1:3000 Caucasians</td>
<td>Airways in the lungs</td>
</tr>
<tr>
<td>Hemoglobinopathies thalassemias</td>
<td>(Structural) defects in the ( \alpha ) or ( \beta ) globin gene</td>
<td>1:600 in certain ethnic groups</td>
<td></td>
</tr>
<tr>
<td>Gaucher’s disease</td>
<td>Defect in the enzyme glucocerebrosidase</td>
<td>1:450 in Ashkenazi Jews</td>
<td>Bone marrow cells, macrophages</td>
</tr>
<tr>
<td>( \alpha_1 ) antitrypsin deficiency</td>
<td>Lack of ( \alpha_1 ) antitrypsin</td>
<td>1:3500</td>
<td>Lung or liver cells</td>
</tr>
<tr>
<td>inherited emphysema</td>
<td></td>
<td>1:3000 males</td>
<td>Muscle cells</td>
</tr>
<tr>
<td>Duchenne muscular distrophy</td>
<td>Lack of dystrophin</td>
<td>1:3000 males</td>
<td></td>
</tr>
<tr>
<td>Disease</td>
<td>Defect</td>
<td>Incidence</td>
<td>Target Cells</td>
</tr>
<tr>
<td>-----------------------</td>
<td>---------------------------------------------</td>
<td>---------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Cancer</td>
<td>Many causes, including genetic and environmental</td>
<td>1 million/year in USA</td>
<td>Variety of cancer cell types, in liver, brain, pancreas, breast, kidney</td>
</tr>
<tr>
<td>Neurological diseases</td>
<td>Parkinson’s, Alzheimer’s and spinal-cord injury</td>
<td>1 million Parkinson’s and 4 million Alzheimer’s patients in the USA</td>
<td>Neurons, glial cells, Schwann cells</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Restenosis, arteriosclerosis</td>
<td>13 million in USA</td>
<td>Arteries, vascular endothelia walls</td>
</tr>
<tr>
<td>Infectious diseases</td>
<td>AIDS, hepatitis B</td>
<td>Increasing numbers</td>
<td>T cells, liver, macrophages</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Autoimmune inflammation of joints</td>
<td>Increasing numbers with aging population</td>
<td></td>
</tr>
</tbody>
</table>
Applications of Gene Therapy in Animals

- Delivery of rhBMP-2 and -7 genes using Gene Therapy to Enhance Bone Healing.
  
  (Southwood, 1996)

- Inherited skin disease: X-linked Hypohidrotic Ectodermal Dysplasia (HED) in Canines treated by using functional EDA gene.
  
  (Wolff, 1997)

- Gene Therapy to cure Traumatic arthritis in Athletic Horses using Equine interleukin-1 receptor antagonist gene.
  
  (Richardson, 1999)

- Citrullinemia in bovines by Systemic administration of a E1-deleted adenoviral vector
  
  (Lee et al., 1999)
Gene therapy to restore **vision** in blind dog by inserting RPE65 gene.

( Aguirre *et al.*, 2001)

**Lysosomal Storage Diseases** (LSD) in mice, dogs and cats is treated by using M6P receptor gene.

( Wolff, 2004)

Gene therapy completely corrected **hemophilia** in laboratory animals (Rat and Dog); clotting factor VIII was used.

(Xu *et al.*, 2005)

Gene therapy cured **deafness** in guinea pigs using Math 1 gene.

(Raphael, 2005)
Corneal gene therapy in the horse and dog using adeno-associated vector serotype 5 (AAV5).

(Basic et al., 2012)

Recently Khatib et al., 2015 have gained success in curing type 1 diabetes mellitus by employing β-cell-targeted adeno-associated virus serotype 8 (AAV8)-based vectors to overexpress interleukin 10. Islets were protected from rejection for at least 120 days.
Factors that have kept GT from becoming an effective treatment for genetic disease?

- Short lived nature of gene therapy
- Immune response
- Problems with Viral vectors
- Insertional mutagenesis
- Multigene disorders
Social and ethical issues

- Accumulation of defective genes in future generation
- Mutation or defective arrangement of genes
- Probability of occurrence of new diseases
- Available to rich and powerful
- Designer babies
- Playing with God
## Gene Therapy in India

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Principle Investigator</th>
<th>Institution/Company</th>
<th>Year</th>
<th>Area of work</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dr. Rita Mulherkar</td>
<td>Advanced Centre for Treatment, Research and Education for Cancer, Mumbai, MH</td>
<td>1998</td>
<td>Gene therapy for head and neck cancer</td>
</tr>
<tr>
<td>2</td>
<td>Dr. Debi Prasad Sarkar</td>
<td>University of Delhi, New Delhi, DL</td>
<td>2002</td>
<td>Development of synthetic and viral delivery systems</td>
</tr>
<tr>
<td>3</td>
<td>Dr. Subrata Banerjee Saha</td>
<td>Institute of Nuclear Physics, Kolkata, WB</td>
<td>2004</td>
<td>Gene therapy for haematological disorders</td>
</tr>
<tr>
<td>4</td>
<td>Dr. Kumaravel Somasundaram</td>
<td>Indian Institute of Science, Bengaluru, KA</td>
<td>2005</td>
<td>Gene therapy for cervical cancer</td>
</tr>
<tr>
<td>5</td>
<td>Mr. Sanjeev Saxena</td>
<td>Actis Biologics Private Limited, Mumbai, KA</td>
<td>2005</td>
<td>Production and analysis of gene therapy products</td>
</tr>
<tr>
<td>6</td>
<td>Dr. Jayandharan Rao</td>
<td>Center for Stem Cell Research, Vellore, TN</td>
<td>2010</td>
<td>Gene therapy for haemophilia and leukemia</td>
</tr>
<tr>
<td>7</td>
<td>Dr. Sanjay Kumar</td>
<td>Center for Stem Cell Research, Vellore, TN</td>
<td>2010</td>
<td>Gene and stem cell therapy for bone disorders</td>
</tr>
<tr>
<td>8</td>
<td>Dr. Everette Jacob</td>
<td>Remington Nelson Vellore Institute of Technology, Vellore, TN</td>
<td>2012</td>
<td>Gene therapy for leukocyte adhesion deficiency</td>
</tr>
<tr>
<td>9</td>
<td>Dr. Rupesh Dash</td>
<td>Institute of Life Sciences, Bhubaneswar, OD</td>
<td>2012</td>
<td>Gene therapy for prostate cancer</td>
</tr>
<tr>
<td>10</td>
<td>Dr. Arkasubhra Ghosh</td>
<td>Narayana Nethralaya, Bengaluru, KA</td>
<td>2013</td>
<td>Gene therapy using AAV vectors</td>
</tr>
</tbody>
</table>

Chodisetty et al., 2014
Gene therapy is a potential treatment designed to supplement, replace or modify the host’s existing DNA.

- Advances in the areas such as identification of genes and understanding the gene expression are required for effective application of gene therapy.

- The use of viral vectors carry the promise of future mediators to improved human and animal health welfare.

- Clinical application of gene therapy could improve the prognosis for human and veterinary patients.
“All inventions start with failures. Gene therapy will be really a boon to medicine in future.”