Therapy of Genetic Disorders

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Control and Prevention Programmes For Genetic Diseases

Genetic Diseases (Control and Prevention)

Increase awareness

Genetic Screening

Appropriate management and consultation programmes

- High risk
- Premarital
- Prenatal
- Neonatal
- General populations

Genetic Counselling

- Patients, families and community
- Clinical staff and premedical personnel
- Health policy makers and administration
**GENETIC COUNSELLING**

- **Counselling** A educational process by which patients or & individuals at risk are given information to understand the nature of the genetic disease, its transmission and the options open to them in management and family planning.
- Genetic counseling - an integral part of the management of patients and families with genetic disorders.
Genetic Counselling

An essential component of health counselling

For control of diseases with partial or complete genetic aetiology

- Single gene disorders
- Chromosomal anomalies
- Multifactorial disorders
- Mitochondrial disorders
Essential Components of Genetic Counselling

- History and pedigree construction
- Clinical Examination
  - History findings
  - Clinical examination findings
  - Radiology findings
  - Laboratory parameter results
  - DNA studies results
  - Others
- Confirmatory diagnosis
- Calculation of recurrence risk
- Counseling
- Available options
- Follow-up
The Genetic Counselling Process

Beneficiaries → Individual or couple seeking counselling →

Reaching accurate diagnosis

Estimation of recurrence risk

Genetic counselling

Decision making

Why?
- Have affected child
- Are carriers
- Have genetic disease in family
- Have recurrent abortions
- High maternal/paternal age
- Exposed to a mutagen/teratogen
- Are consanguineous

Counselling elements

- Family history
- Physical/clinical examination
- Cytogenetic studies/radiology
- Laboratory/DNA analysis

- Family pedigree
- Applying various risk calculation methods:
  - Bayesian
  - Mendels

- Available options
- Risk calculations
- New developments, etc.
- Disease course
- Treatment availability

Decision making

- Knowledge of disease recurrence
- Non-directive
- Available options
- Family pressure
- Religious beliefs
- Social status
- Economic status
- Community influence
Types of Genetic Testing

1. Carrier testing
2. Premarital Screening
3. Neonatal testing:
4. Prenatal diagnosis
5. Preimplantation diagnosis
6. Cell and cell free DNA in Maternal blood
7. Therapy of Genetic Disorders
Current Therapy of Genetic Disorders

- Preventive
- Metabolic Manipulation
- Gene Product Replacement
- Cell or Organ Transplantation
- Gene Therapy
Therapy of Genetic Disorders

• Preventive Therapy
  • Preventive screening
    • Neonatal screening
    • Population screening
  • Prenatal diagnosis
  • Preimplantation diagnosis
Therapy of Genetic Disorders

- **Metabolic Manipulation**
  - **Dietary Restriction**
    - (Lactose restriction for Lactase deficiency; phenylalanine restriction for phenylketonuria)
  - **Dietary Supplementation**
    - (Biotin for Biotinidase deficiency, Starch for G-6-P deficiency)
  - **Chelation and enhanced excretion**
    - (Copper chelation for Wilson Disease)
  - **Metabolic inhibitors**
    - (allopurinol for gout, Statins for hypercholesterolemia)
Therapy of Genetic Disorders

• Gene Product Therapy
  ➢ Hormone, protein or enzyme replacement
    ▪ Hormone supplementation:
      ▪ Hypothyroidism: Thyroid hormones
      ▪ Congenital adrenal hyperplasia: Cortisol
      ▪ Growth hormone
    ▪ Hemophilia: Clotting factors
    ▪ Diabetes: Insulin
    ▪ Enzyme replacement
      ▪ Gauchers: Beta glucosidase
      ▪ Pompe: Alpha glucosidase
      ▪ SCID: Adenosine deaminase
Examples of Current Enzyme Therapy

• Current FDA approved enzyme replacement therapy
  - Adenosine deaminase deficiency (SCID)-
    • Severe combined Immunodeficiency
    • No targeting to cells, but removal of metabolites from plasma
  - Several Lysosomal Storage Disorders
    • Genetic deficiency of Lysosomal Enzymes
    • Therapy: Targeting of deficient enzyme to lysosomes
ENZYME REPLACEMENT THERAPY FOR LYSOSOMAL STORAGE DISEASES

- **Gaucher Disease** Approved 1991
- **Fabry Disease Approved** 2001 (EU), 2003 (US)
- **MPS I Approved** 2003 (EU & US)
- **MPS VI Approved** 2005 (US& EU)
- **MPS II Approved** 2006 (US)
- **Pompe Disease Approved** 2006 (US & EU)
- **Niemann-Pick B Disease** Phase 1 Trial Underway
Current Enzyme Therapy of Lysosomal Disorders with Intracellular Replacement of Enzyme:

Currently “standard of care”
Gauchers Disease (beta glucosidase; non neuronopathic)

Current Clinical Trials:
Glycogen Storage Disease Type II (acid maltase)

Fabry Disease (alpha galactosidase)

Hurler Disease (alpha iduronidase)

Hunter Disease (iduronate sulfatase)
Therapy of Genetic Disorders

• Cell or Organ Transplantation

Cells:
- Bone marrow,
- Stem cells: Embryonic, adult SC Mesenchymal and Peripheral

Organs
- Kidney: Fabry Disease
- Liver: Tyrosinemia
STEM CELL THERAPY
Potential of Stem Cells

- **Totipotent (total):**
  - Total potential to differentiate into any adult cell type
  - Total potential to form specialized tissue needed for embryonic development

- **Pluripotent (plural):**
  - Potential to form most or all differentiated adult cell types

- **Multipotent (multiple):**
  - Limited potential
  - Forms only multiple adult cell types
    - Oligodendrocytes
    - Neurons
Properties of Human Embryonic Stem Cells in Culture

- **Pluripotent** – able to form any of ~200 different types of cells of the body
- **Self-renewing in vitro** – can propagate or proliferate indefinitely in the undifferentiated state
- Express the telomerase enzyme and *Oct4* (a master regulator of ESC pluripotency)
- Maintain normal chromosome structure and complement even after long periods in culture (unlike many other tissue culture cell lines)
Adult Stem Cells

• Adult or somatic stem cells have unknown origin in mature tissues
  ▪ Unlike embryonic stem cells, which are defined by their origin (inner cell mass of the blastocyst)
Embryonic vs Adult Stem Cells

- Totipotent
  Differentiation into ANY cell type
- Known Source
- Large numbers can be harvested from embryos
- May cause immune rejection

- Multi or pluripotent
  Differentiation into some cell types, limited outcomes
- Unknown source
- Limited numbers, more difficult to isolate
- Less likely to cause immune rejection, since the patient’s own cells can be used
Magnetic Positive Selection

Stem Cell Markers

- c-Kit
- Oct4 (ATGCAAAT)
  POU Family Protein
- CD34
- CD38
- Cd44
- CD133
- Nestin

Magnetic labeling
HSC Gene Therapy Timeline

**MILESTONES**

- **1950**: First BMT (identical twins) by Thomas et al. 1959
- **1960**: First virus-mediated gene transfer by Rogers and Pfuderer 1968
- **1970**: SCID is first disease treated with matched unrelated donor HSCT by Horowitz et al. 1975
- **1980**: First application of gene-marked cells in humans by Rosenberg et al. 1989
- **1990**: G-CSF mobilized blood as source for HSCT by Molineux et al. 1990
- **2000**: Non-myeloablative conditioning regimen by Giralt et al. 1997
- **2010**: First approval of a gene therapeutic (Glybera®) in the EU by Gaudet et al. 2012

**CLINICAL TRIALS**

  - Initiation of clinical trials with gene-modified HSC

- **SIN-gammaretroviral**: ADA-SCID 1990
  - First gene therapy clinical trial (gene-modified T cells)

- **SIN-lentiviral**: SCID-X1 2010, X-CGD 2013
  - X-ALD 2005
  - β-thalassemia 2007
  - WAS, MLD 2010
  - ADA-SCID 2012
  - X-CGD 2012/13

**Safety improved vector design**

**Development of advanced assays for risk assessment**
Stem Cell Therapy Challenges

- Ethical considerations for ESC research
- Safety challenges – Use of ESCs or differentiated cells derived from ESCs for therapy? Considerations to avoid tumor formation. Immune system challenges to avoid rejection of foreign cells.
- Understanding the basic mechanisms that underlie stem cell biology
Summary:

- Stem cell therapies offer regenerative prospects for numerous human diseases.
- Stem cells are capable of renewal and differentiation.
- Stem cells are derived from numerous sources and have different potency capacities.
- Adult stem cells (ASCs) have been detected in numerous tissues.
- Considerable debate surrounds the use of embryonic stem cells, Adult stem cells may offer similar prospects for therapy as do as ESCs, yet a complete understanding of stem cell applications will require a basic understanding of differentiation and renewal mechanisms in ASCs and ESCs as well.
GENE THERAPY

- Replacement Therapy
- Gene transfer
- Gene manipulation
- Cloning
- Stem cell
GENE

Gene Product

Metabolic Effect

Functional Effect

Structural Effect
Disease Characteristics
Currently Ideal for Gene Therapy

- Lethal disorder
- Course not highly variable
- Reversible
- No universal therapy
- Gene cloned
- No tissue specificity or regulation
- Bone marrow cells involved
Gene Therapy Strategies

- Interference with gene products
- Replacement of a missing or defective gene
- Introduction of gene(s) to influence cellular process
Considerations for Gene Therapy

- State of the art of genetic engineering
- State of the art of manipulation of cells and organs
- Disease characteristics
Gene Replacement strategy

- Applies to diseases caused by single gene defects
- Transfer of a functional copy of the defective or missing gene
- Examples: enzyme deficiencies
Gene Replacement Strategy

To apply this strategy, three requirements must be met:

1. The specific gene defect must be known
2. A functional copy of the gene must be available
3. Target cells must be available and amenable to transfection methods resulting in long-term expression
State of the Art of Genetic Engineering

• **Ideal**
  - Replace defective gene with normal (site specific insertion)
  - Target vector containing the gene to damaged cell
  - *In vivo* administration safe, effective and permanent (integration into DNA but not at oncogenic sites)
  - Vector contains all regulatory elements

• **Current**
  - Site specific insertion very early and experimental
  - No current trial incorporates all of the ideal requirements
# Gene Replacement Strategy

<table>
<thead>
<tr>
<th>Gene with defect</th>
<th>Disease/Disorder</th>
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<tbody>
<tr>
<td>Adenosine deaminase (ADA)</td>
<td>SCID</td>
</tr>
<tr>
<td>a-1-antitrypsin</td>
<td>Emphysema</td>
</tr>
<tr>
<td>CF transmembrane regulator</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>Clotting factor VIII</td>
<td>Hemophilia A</td>
</tr>
<tr>
<td>Clotting factor IX</td>
<td>Hemophilia B</td>
</tr>
<tr>
<td>b-chain of hemoglobin</td>
<td>Sickle cell anemia</td>
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Variables in Current Gene Therapy Trials

- Vector for delivery of gene
- *Ex vivo vs In vivo* administration
- Permanent integration into DNA vs transient expression
- Incorporation of regulatory elements
Gene Transfer: Types of Vectors

- **RNA viruses (Retroviruses)**
  1. Murine leukemia virus (MuLV)
  2. Human immunodeficiency viruses (HIV)
  3. Human T-cell lymphotrophic viruses (HTLV)

- **DNA viruses**
  1. Adenoviruses
  2. Adeno-associated viruses (AAV)
  3. Herpes simplex virus (HSV)
  4. Pox viruses

- **Non-viral vectors**
  1. Liposomes
  2. Naked DNA
  3. Liposome-polycation complexes
  4. Peptide delivery systems
Ideal Viral Vectors

- Replication defective
- Accommodates large inserts
- High titer with broad cell range
- High level of expression of inserted gene
- Unique promoters
  - Tissue specific vs universal
  - On/off switch; controllable expression
- Non-toxic
Types of Somatic Gene Transfer

- **Ex vivo**
  - Gene or expression vector carrying the gene is inserted into explanted or cultured cells which are then transplanted into the patient

- **In vivo**
  - Gene or expression vector carrying the gene is administered directly to the patient
Ex vivo gene therapy

1. The genetic material is first transferred into the cells grown in vitro

2. Controlled process;
Genetically altered cells are selected and expanded; more manipulations

3. Cells are then returned back to the patient
In vivo and ex vivo gene therapy concepts
Proposed concept of designer nuclease-mediated correction of patient-specific iPSC for autologous transplantation.
Gene therapy could be very different for different diseases

Gene transplantation
(to patient with gene deletion)

Gene correction
(To revert specific mutation in the gene of interest)

Gene augmentation
(to enhance expression of gene of interest)
Barriers to successful gene therapy:

1. Vector development
2. Corrective gene construct
3. Proliferation and maintenance of target cells
4. Efficient transfection and transport of DNA to nucleus for integration into genome
5. Expansion of engineered cells and implantation into patient
Creation of recombinant DNA molecules in vitro

plasmid cloning vector
SCID treatments

Life in germ-free environment

Bone-marrow transplantation

Enzyme replacement therapy

VERY expensive; not a cure; temporary effect

GENE THERAPY
“Successful” Gene Therapy for Immunodeficiency Diseases: 2005

- Retroviral vector used despite major disadvantages

- Over 14 patients with X-linked severe combined immunodeficiency of 3 different types have been treated successfully

- Oncogenic insertion in two of 14 children—leukemia

- X-linked SCID trials suspended but now reinstituted

- ~8 patients with ADA deficiency treated
SCNT: Somatic Cell Nuclear Transfer

• SCNT is a method used for:
  ▪ Reproductive cloning such as cloning an embryo
  ▪ Regenerative cloning to produce “customized” stem cells & overcome immune rejection

• Blastula stage cannot continue to develop in vitro
  ▪ It must be implanted into surrogate mom
  ▪ Surrogate mom is just a container that provides protection & chemical signals necessary for development
Protein Production in Transgenic Sheep

YFG = Your Favorite Gene

**Diagram:**

1. Sheep ovum
2. DNA is injected into pronucleus
3. Implant into foster mother
4. Transgenic progeny are identified by PCR
5. Expression of YFG is restricted to mammary tissue
6. Obtain milk from transgenic animals
7. YFG product is secreted into milk
8. Fractionate milk proteins
9. Pure YFG product
Spectrum of Gene expression

Cancer Gene Therapy

1. Oncogene inactivation
2. Augmentation of TSG
3. Cell targeted suicide-pro-drug to toxic metabolite by transfer of converting enzyme gene into tumor cells
4. Chemoprotection - transfer of MDR (Multi Drug Resistance) gene into bone marrow cells to decrease effect of cytotoxic agents
Drug Activation Gene Therapy for Cancer

*Discriminating between normal and cancer cells by selective drug activation.*