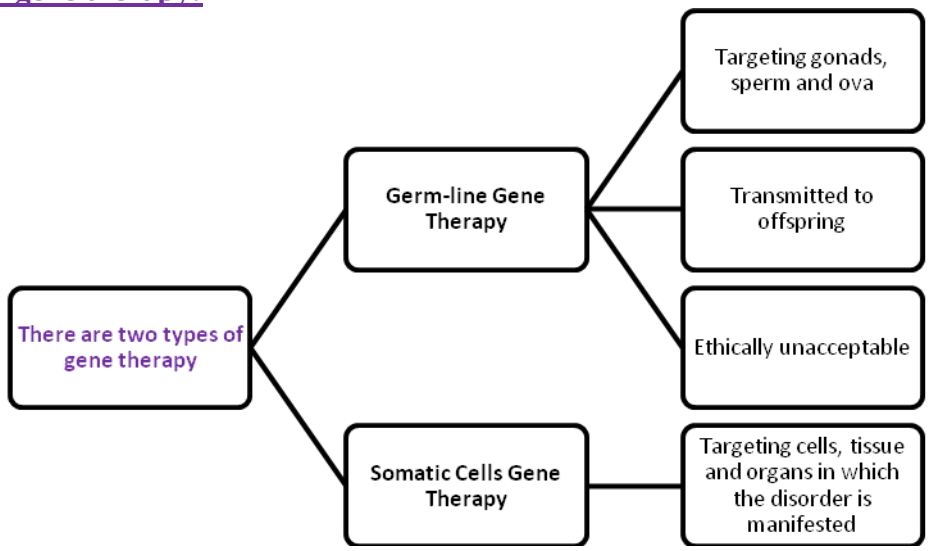
Unit IX – Problem 3 – Genetics: Gene Therapy

- **What is a gene?**
  - A sequence of chromosomal DNA that is required for production of a functional protein (it contains both introns and exons).
- **What is gene therapy:**
  - It is the treatment of human diseases by transferring genetic material into a person's cell to fight/ prevent the disease.
- **Types of gene therapy:**



- **Is gene therapy applied in all diseases?** → no, there are requirements:
  - **Disease:**
    - ✓ The disease must be serious.
    - ✓ Incurable with conventional treatment.
    - ✓ Chronic (requires life-long treatment).
  - **Gene:**
    - ✓ The gene responsible for the disease must be identified.
    - ✓ Copies of the gene can be made in the lab.
    - ✓ Role of protein encoded by the gene is known.
  - **Target organ:**
    - ✓ Readily accessible.
    - ✓ Long survival time.
    - ✓ Ability to replicate itself.
  - **Ethical approval.**
- **Diseases which are targeted by gene therapy:**
  - **Inherited disease with single-gene defect:**
    - ✓ Severe Combined Immunodeficiency disease (SCID).
    - ✓ Duchenne muscular dystrophy.
    - ✓ Hemophilia.
    - ✓ β-thalassemia.
    - ✓ Cystic fibrosis.
  - **Poly-genic (multiple gene-defects are responsible for the disease) or non-inherited diseases:**
    - ✓ Infectious diseases: HIV and hepatitis-C
    - ✓ Rheumatoid Arthritis (RA).
    - ✓ Cancer (it is the most targeted disease in gene therapy).
    - ✓ Cardiovascular diseases.
- **Gene therapy – step by step:**
  - **Identify the gene which is responsible for the disease.**
  - **Make copies of the normal gene.**

- **Insert the copies into a vector (mostly virus):**
  - ✓ Remove the viral genome and then insert the gene of interest.
- **Infect affected cells with the vector:**
  - ✓ Normal gene is carried into the nucleus.
  - ✓ The DNA may integrate into the genome.
- **Activate the gene (transcription & translation take place).**

- **There are two approaches of gene delivery:**
  - **In vivo approach**: injection of the vector into the body and specifically target affected cells.
  - **Ex vivo approach**: delivering the gene to cells while they are outside the body.
- **Strategy of gene therapy:**
  - **Loss of function mutation**: insert a copy of normal gene.
  - **Gain of function mutation**: replace with the normal gene.
  - **Cancer gene therapy:**
    - ✓ Stimulation of natural killing of tumor cells (IL-2).
    - ✓ Anti-angiogenic genes (inhibiting VEGF).
    - ✓ Supply tumor suppressor genes (p53).
    - ✓ Inhibition of oncogenic proteins (such as bcr-abl).
    - ✓ Transporting anti-cancer genes selectively into cancer cells using nanotechnology (magic bullet!).
- **What are the characteristics of an ideal vector?**
  - Has an adequate carrying capacity.
  - Easy to produce in high concentrations.
  - Targeting specific tissues.
  - Stable.
  - No immune response, non-inflammatory and non-toxic.
  - High efficiency.
  - Long duration of expression.
- **Methods of gene delivery:**
  - **Transduction**: viral-mediated gene transfer.
  - **Transfection**: non-viral mediated gene transfer.
- **Types of vectors:**
  - **Viral vectors (transduction):**
    - ✓ Retroviruses: these are RNA viruses encoding for reverse transcriptase enzyme. They are used in ex vivo gene therapy.
      - ❖ *Advantages:*
        - ➢ DNA integrated into host genome.
        - ➢ Non-immunogenic and non-toxic.
      - ❖ *Disadvantages:*
        - ➢ Small insert size.
        - ➢ Infect only dividing cells.
        - ➢ Risk of insertional mutagenesis which leads to activation of oncogenes.
    - ✓ Lentiviruses:
      - ❖ *Advantages:*
        - ➢ Infect dividing and non-dividing cells.
        - ➢ Easier to culture than retroviruses.
      - ❖ *Disadvantages:*
        - ➢ Risk of insertional mutagenesis which leads to activation of oncogenes.

- ✓ Adenoviruses (these are DNA viruses):
  - ❖ *Advantages:*
    - ➢ No risk of insertional mutagenesis.
    - ➢ Large.
    - ➢ Infect dividing and non-dividing cells.
  - ❖ *Disadvantages:*
    - ➢ Short duration of expression.
    - ➢ Immunogenic with high toxicity (might lead to death).
- ✓ Adeno-associated viruses:
  - ❖ *Advantages:*
    - ➢ Inserted into chromosome 19.
    - ➢ Infect wide variety of cells.
    - ➢ Long duration of expression.
    - ➢ Low immune response.
  - ❖ *Disadvantages:*
    - ➢ Carry small insert size.
- ✓ Herpes virus:
  - ❖ *Advantages:*
    - ➢ Infects nervous tissue.
  - ❖ *Disadvantages:*
    - ➢ Neurotoxicity.

- **Non-viral vectors (transfection):**
  - ✓ Types:
    - ❖ Liposomes.
    - ❖ Direct injection of naked DNA.
    - ❖ Receptor-mediated endocytosis.
    - ❖ Oligonucleotides.
  - ✓ Advantages:
    - ❖ Carrying large insert size.
    - ❖ Targeting specific cells.
    - ❖ Non-immunogenic.
    - ❖ No risk of insertional mutagenesis.
  - ✓ Disadvantages:
    - ❖ Low efficiency.
    - ❖ Transient expression (short-term expression).
    - ❖ Degradation of protein-DNA conjugate (by lysosomes of the cell).

- **What are the problems of gene therapy?**
  - Short-lived nature of gene therapy.
  - Immune response to vector.
  - General toxicity of viral vectors.
  - Insertional mutagenesis.
  - Multi-gene disorders are hard to treat because you need to introduce more than one gene.
  - Contamination of germ-line cell.
  - Expensive.
- **Current status of gene therapy**: mostly experimental and has not proven very successful in clinical trials.
- **Gene doping**: non-theraputic use of cells, genes, genetic elements of or the modulation of gene expression having the capacity to improve athletic performance.