BHARATHIDASAN UNIVERSITY

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(5 Year Integrated Program)

Course Title : Genomics
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Unit-V
Gene Therapy

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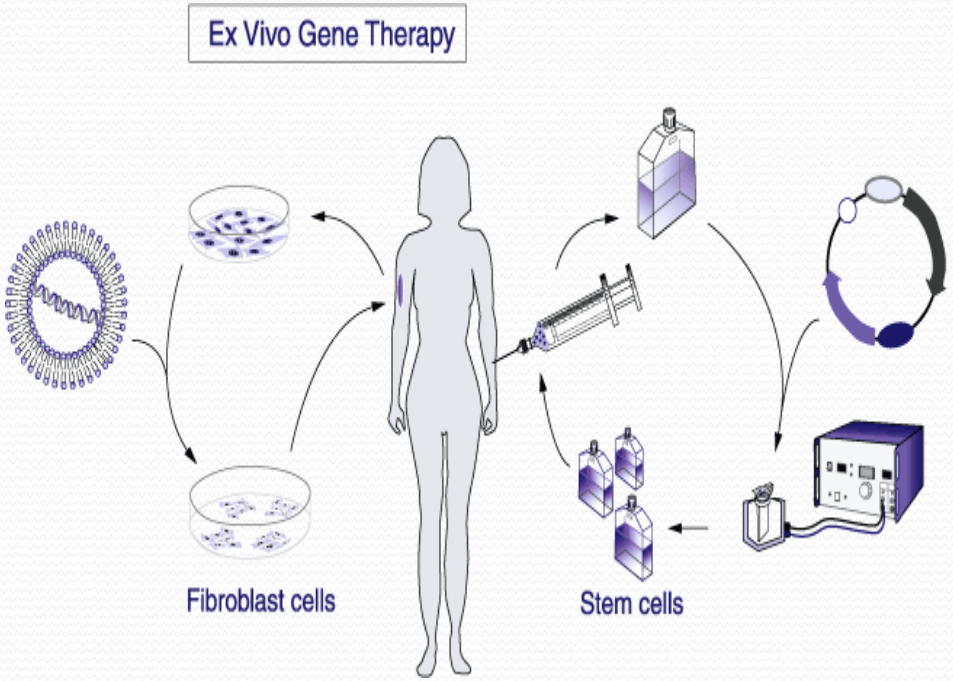
Gene therapy

- It is a technique in which, a "normal" gene is inserted into the genome to replace an "abnormal," disease-causing gene.
- Two types of Gene therapy are in practice.
  - Germline gene therapy
    - germ cells, i.e., sperm or eggs, are modified by the introduction of functional genes, which are ordinarily integrated into their genomes.
  - Somatic cell gene therapy
    - the therapeutic genes are transferred into the somatic cells of a patient.
- The first gene therapy was performed on September 14th, 1990

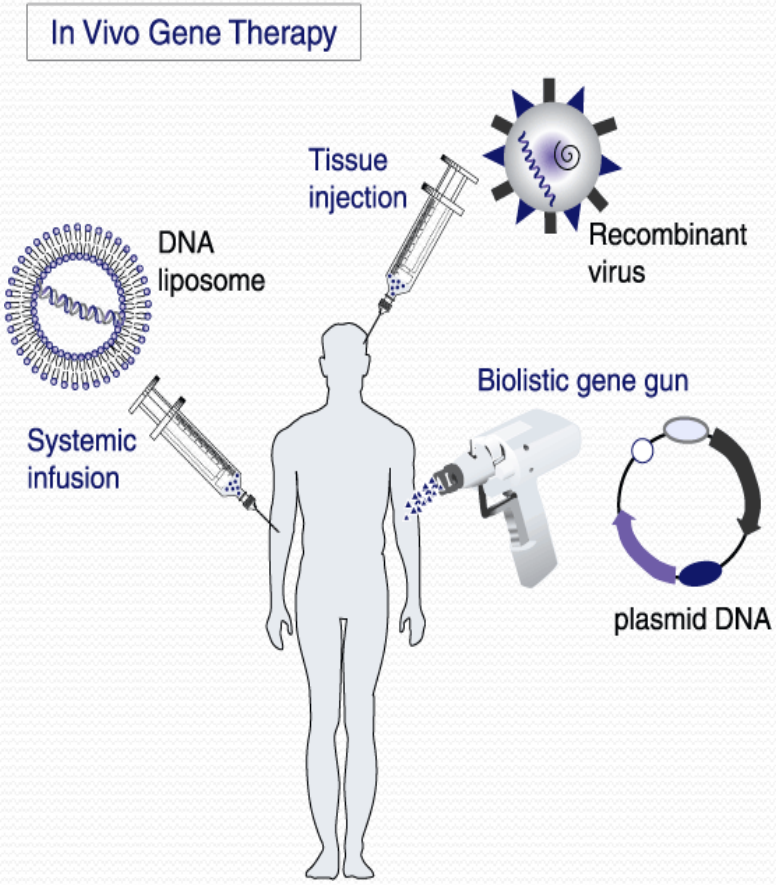
Ex-Vivo Gene therapy

- Ex-vivo gene therapy is performed by transfecting or infecting patient-derived cells in culture with vector DNA and then reimplanting the transfected cells into the patient. Two types of ex-vivo gene therapies under development are those directed at fibroblasts and hematopoietic stem cells.



In vivo Gene therapy

- In vivo gene therapy requires that the gene transfer vector be delivered in a cell-type selective manner, either through direct tissue injection, or perhaps someday, by receptor-mediated processes.



Gene Delivery System

- There are two types of Gene delivery system
  - Viral and
  - Non-viral

- Viruses are obligate intra-cellular parasites, designed to infect cells, often with great specificity to a particular cell type.

- Non-viral methods present certain advantages over viral methods, with simple large scale production and low host immunogenicity.

Viral vectors

- Viral vectors tend to be very efficient at transfecting their own DNA into the host cell.
- Replicate by inserting their DNA into a host cell.
- Gene therapy can use this to insert genes that encode for a desired protein to create the desired trait
- Four types of viral vectors are used
  - Retrovirus
  - Adenovirus
  - Adeno-associated virus
  - Herpes Simplex virus

Retrovirus

- Retroviruses are a class of enveloped viruses containing a single stranded RNA molecule as the genome.
- Following infection, the viral genome is reverse transcribed into double stranded DNA, which integrates into the host genome & is expressed as proteins.
- The viral genes are replaced with the transgene of interest .
- The essential regions include the 5' & 3' LTRs & the packaging sequence lying downstream of the 5' LTR.
- Transgene expression can either be driven by the promoter/enhancer region in the 5' LTR, or by alternative viral or cellular promoters.

- Advantages
  - Transgene integrated into the genome is stable.
  - Includes region that can stably express the therapeutic gene.

- Disadvantages
  - The target cells should be dividing.
  - Integration can activate a proto-oncogene.
  - May evoke immune response

Adenovirus

- Adenoviruses are non-enveloped viruses containing a linear double stranded DNA genome.
- The life cycle does not normally involve integration into the host genome, rather they replicate as episomal elements in the nucleus of the host cell.
- There are four early transcriptional units (E1, E2, E3 & E4), which have regulatory functions, & a late transcript, which codes for structural proteins.
- Vectors contain only the inverted terminal repeats (ITRs) & a packaging sequence around the transgene, all the necessary viral genes being provided in trans by a helper virus. (gutless vectors)

- Advantages
  - Can target non-dividing cells.
  - Does not activate other genes.

- Disadvantages
  - Majority will be degraded.
  - Promoter gets inactivated soon.
  - May evoke immune response

Adeno-associated virus

- Adeno-associated viruses (AAV) are non-pathogenic human viruses depend on a helper virus, usually adenovirus, to proliferate.
- The wild type genome is a single stranded DNA molecule, consisting of two genes Rep and Cap.
- Integrates into the host genome.
- The rep & cap genes are replaced by the transgene of interest.
- The helper virus provides the Rep and Cap genes.

- Advantages
  - Can target both dividing & non-dividing cells.
  - Prolonged transgene expression.

- Disadvantages
  - Cant replicate without helper virus.
  - Can activate other genes.
  - May evoke immune response

Herpes Simplex Virus

- Herpes simplex virus type 1 (HSV-1) is a human neurotropic virus, used as a vector for gene transfer to the nervous system.
- The viral genome is a linear double stranded DNA molecule.
- Two basic approaches have been used for production of HSV-1 vectors, namely amplicons & recombinant HSV-1 viruses.
- Amplicons consists of col E1 ori (an Escherishia coli origin of replication), OriS (the HSV-1 origin of replication), HSV-1 packaging sequence, the transgene under control of an immediate-early promoter & a selectable marker.
- Recombinant viruses are made replication deficient by deletion of one of the immediate-early genes ,which is provided in trans.

- Advantages
  - Able to infect neurons.
  - Does not integrate into the genome.
  - Less pathogenic.

- Disadvantages
  - Toxic to neurons in culture.
  - Promoter gets inactivated soon.
  - May evoke immune response.

Non-Viral vectors

- Direct introduction of therapeutic DNA to the target cell.
  (Naked DNA)

- Creation of artificial lipid sphere with aqueous core, liposome that can Carry the therapeutic DNA through membrane .
  (Cationic lipids)

- Chemically linking DNA to molecule that will bind to special cell receptors which facilitates DNA to be engulfed by cell membrane.
  (ploymers)

Naked DNA

- This is the simple method used to transfect Dna into the cells.
- Carried out by simple intramuscular injection.
- Expression is low when compared with other methods.
- Application of electroporation and “Gene gun” facilitates more efficiency.

Cationic Lipids

- To improve DNA delivery and protection from damage the DNA is covered with lipids in an organised structure called Lipoplex.
- Cationic lipids, due to their positive charge, condense negatively charged DNA molecules so as to facilitate the encapsulation of DNA into liposomes.
- It also enhances the stability of Lipoplex.
- Major route of uptake is believed to be endocytosis.

- Advantages
  - Commomly used for gene transfer in cancer.
  - Useful in treating genetic diseases.

- Disadvantages
  - Dose dependent toxicity is seen.
  - Endosomes formation more susceptible to Lysosomal degradation.

Polymers

- Complexes of polymers with DNA are called polyplexes.
- Most polyplexes consist of cationic polymers and their production is regulated by ionic interactions.
- Polyplexes release DNA into the cytoplasm through cotransfection with endosome-lytic agents.
- Polyethylenimine, chitosan and trimethylchitosan does not require endosome-lytic agents since they have their own method of Endosomal disruption.

Prodrug activation therapy

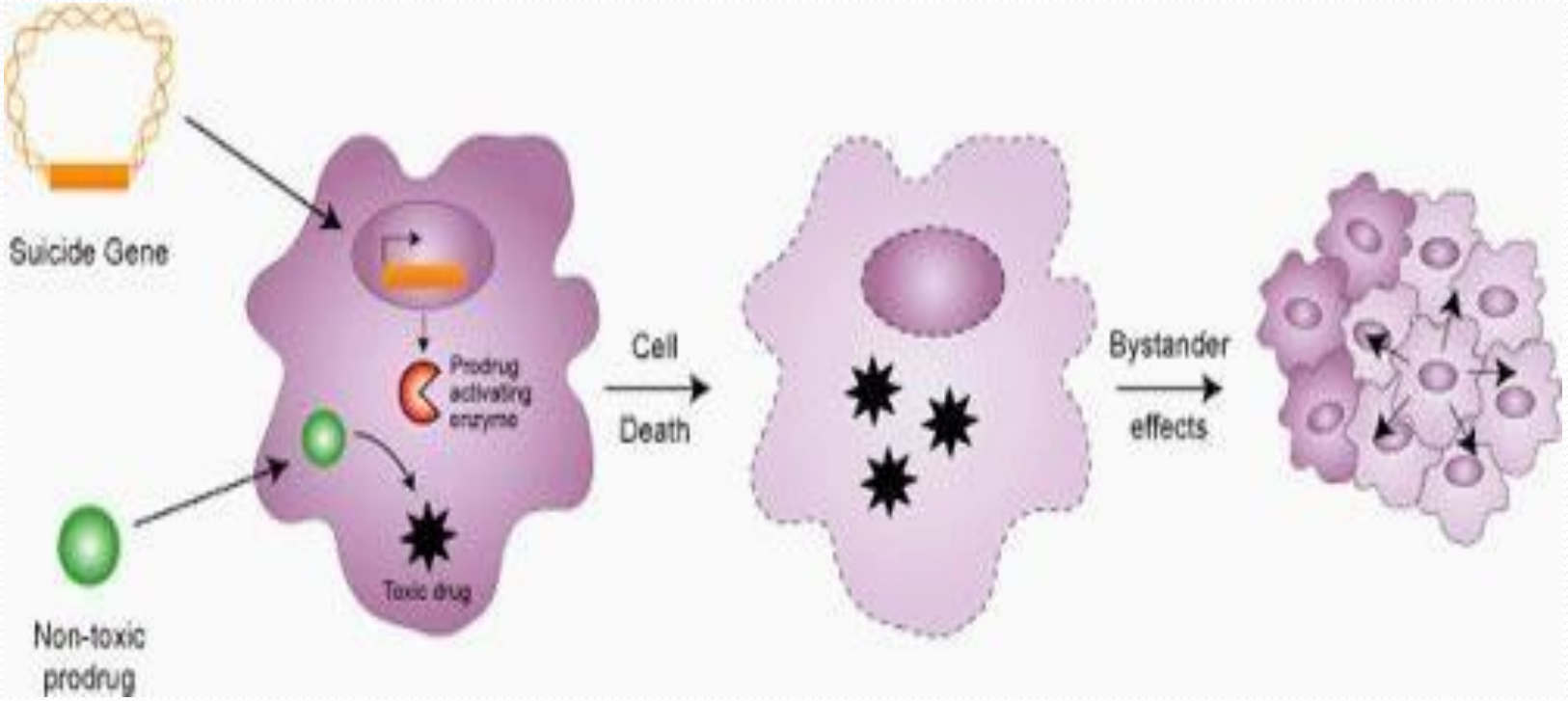
- In cancer chemotherapy , insufficient therapeutic index ,lack of specificity, leads to emergence of drug resistance cell subpopulations.
- One approach to improve the specificity of chemotherapy could be enzyme-activating prodrug therapy in which the transgenes encode enzymes that convert specific, less-toxic prodrugs to toxic metabolites in the tumour cells.(GDEPT)

- In the first step, the gene for a foreign enzyme (viral, bacterial or yeast) is delivered and targeted in a variety of ways to the tumor.

- In the second step, a far less-toxic prodrug is administered systemically and converted to its active cytotoxic substance.

- The drug also exhibits Bystander effects which kills neighboring tumor cells also.

- In addition, dying cells can induce host immune responses mediated by natural killer (NK) cells and T-cells.



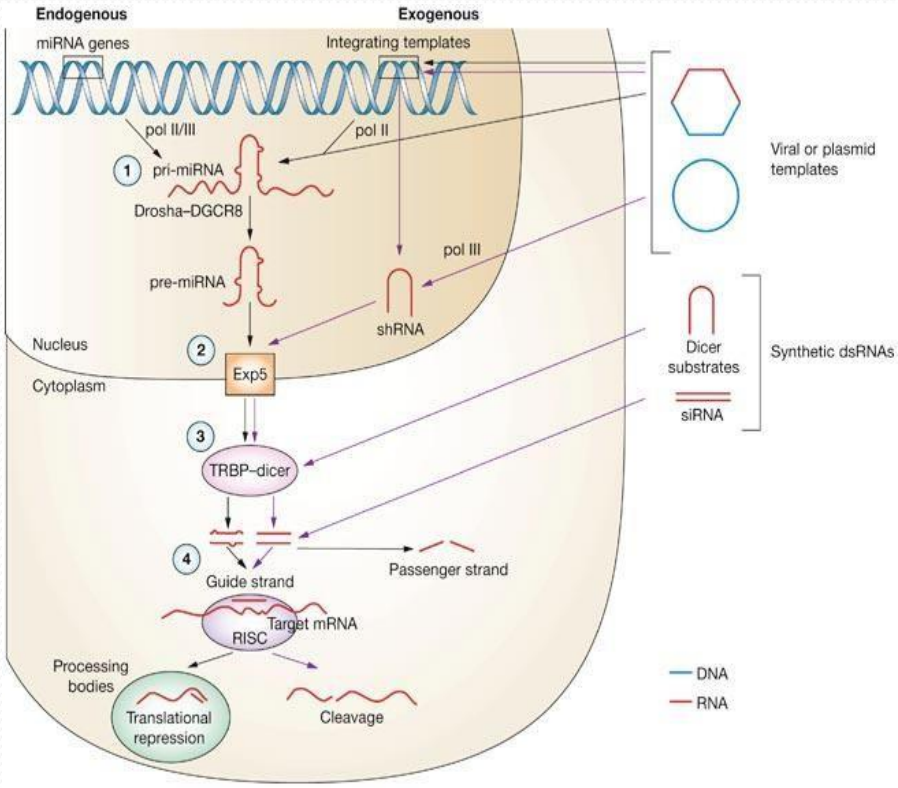
Advantages of GDEPT

- Expressed exclusively in tumor cells.
- Concentration sufficient to activate prodrug.
- Catalytic activity – adequate for prodrug activation under physiological condition.
- Bystander effect
- Less cytotoxic, stable.
- Substrate for activating enzyme under physiological conditions.
- Diffuse --- tissue interstitium.
- Good pharmacological and pharmacokinetic properties.
- Drug formed –highly diffusible or taken up by cells.

RNA Interference (siRNA & miRNA)

- RNAi is a biological phenomenon in which a strand of RNA cause the destruction of an mRNA with that the gene's message is effectively destroyed.
- Andrew Z. Fire and Craig C. Mello were awarded noble prize for their discovery of RNA interference – gene silencing by double stranded RNA in 2006.
- RNAi gene therapy has the opposite effect. When used as gene therapy, RNAi turns off genes that are overactive in such diseases as cancer.
- Silencing was specific for a mRNA homologous to the dsRNA-other mRNAs were unaffected.

- Most endogenous miRNA genes are transcribed by RNA polymerase II before nuclear processing by the Drosha–DGCR8 complex.
- Once in the cytoplasm, dsRNAs still requires processing by TRBP–Dicer.
- All dsRNAs converge in loading the guide strand into RISC.
- Exogenous siRNAs do not need Dicer processing.
- Endogenous miRNAs mostly lead to translational repression, and exogenous dsRNAs to target cleavage



- **DGCR8: DiGeorge syndrome critical region 8**
- **Exp5: exportin-5**
- **RISC: RNA-induced silencing complex**
- **TRBP, trans-activation-responsive RNA binding protein.**

- Advantages
  - Gene silencing was efficient and specific.
  - Low amounts sufficient for silencing.
  - Protects against viral infections.

- Disadvantages
  - High doses may be fatal.
  - siRNAs may evoke immune response.
  - May shut down normal genes.

Gene therapy for diseases

- Gene therapy has recently emerged as a effective therapy that promises to overcome several drawbacks in the available therapeutic approaches.
- Clinical trails to treat various genetic disorders using gene therapy is progressing with some promising outcomes.
- Recently gene therapy for various diseases like cystic fibrosis, multiple sclerosis, Parkinson's disease, Alzheimer's disease were established.

Coronary heart disease

- Coronary heart disease is characterised by gradual narrowing of the lumen in the arteries and subsequent reduction in blood flow to the heart.

- BIOBYPASS, a standard modified adenovector with a standard cytomegalovirus (CMV) promoter carrying the transgene encoding vascular endothelial growth factor injected along CABG yielded promising results.

Cystic fibrosis

- Cystic fibrosis is a heterogeneous recessive genetic disorder with features that reflect mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene.

- Gene therapy involves inhaling a spray that delivers normal DNA to the lungs.

Multiple sclerosis

- MS is known to be a chronic inflammatory disease in which patients exhibit elevated levels of proinflammatory cytokines.
- Anti-inflammatory cytokines such as IFN-β, IL-10, or IL-4 will be administered for treatment using gene delivery systems.
- Researchers usually engineer either T cells or DC ,cultured in a petri dish, to produce various therapeutic compounds, usually anti-inflammatory cytokines and then delivered to the host cell.

Parkinson’s disease

- Parkinson's disease is a movement disorder caused by a progressive depletion of the brain chemical dopamine.
- In Parkinson's disease , GABA is reduced in an area of the brain called the subthalamic nucleus.
- Gene therapy involves infusion of vector carrying GAD, glutamic acid decarboxylase gene. This enzyme is critical in controlling GABA.

Huntington’s chorea

- Huntington's chorea is a disease that is a progressive, degenerative neurological disorder that slowly takes away a persons ability brain,to walk, talk, and reason.
- Caused by a mutation in the gene called “huntingtin” that leads to a toxic accumulation of abnormal protein in the brain.
- An *exvivo* gene therapy involves delivery of genetically modified BHK cells(hamster kidney cells) that delivers ciliary neurotrophic factor (CNTF).
- CNTF proved to protect neurons.

Alzheimer’s disease

- Alzheimer's disease is the most common form of dementia commonly recognized as memory loss.
- CERE-110 is a adeno-associated viral delivery of Nerve Growth Factor during surgery into a part of the brain affected by Alzheimer's disease.
- The gene will instruct brain cells to produce more of a protein, NGF which helps nerve cells survive and function properly.