Gene Therapy

Using Viral and Non-Viral Vectors to Deliver Therapeutic Genes to the Human Body

By Jay Bhat

Introduction

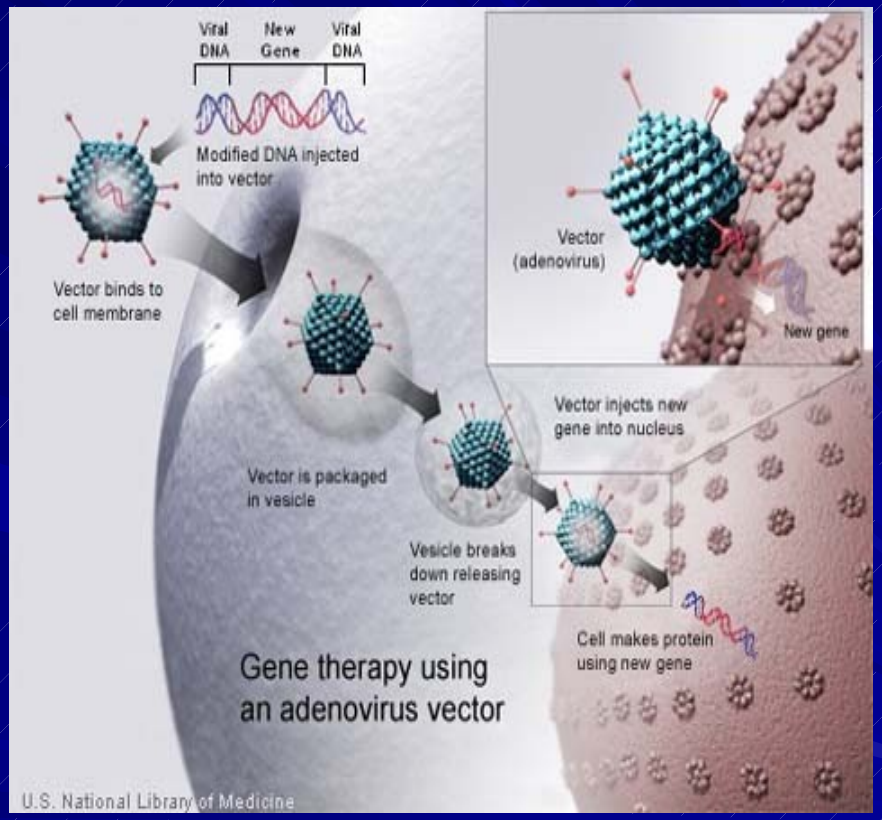
- 2 Types
  - Germline Gene Therapy (Theoretical)
  - Somatic Gene Therapy (Clinical)
- Goal: Correct a Genetic Disorder by Altering Genetic Data
- Limitations: Actual Genetic Defect can't be Changed (DNA in all Cells)
  - Still Limit the Defective Gene's Expression

Treatment of Genetic Disorder

- Expression of healthy gene over a defective gene
- Deactivating an improperly functioning mutated gene
- Introducing a counteracting gene to fight manifestations of a disease
- Altering the regulation of a certain gene

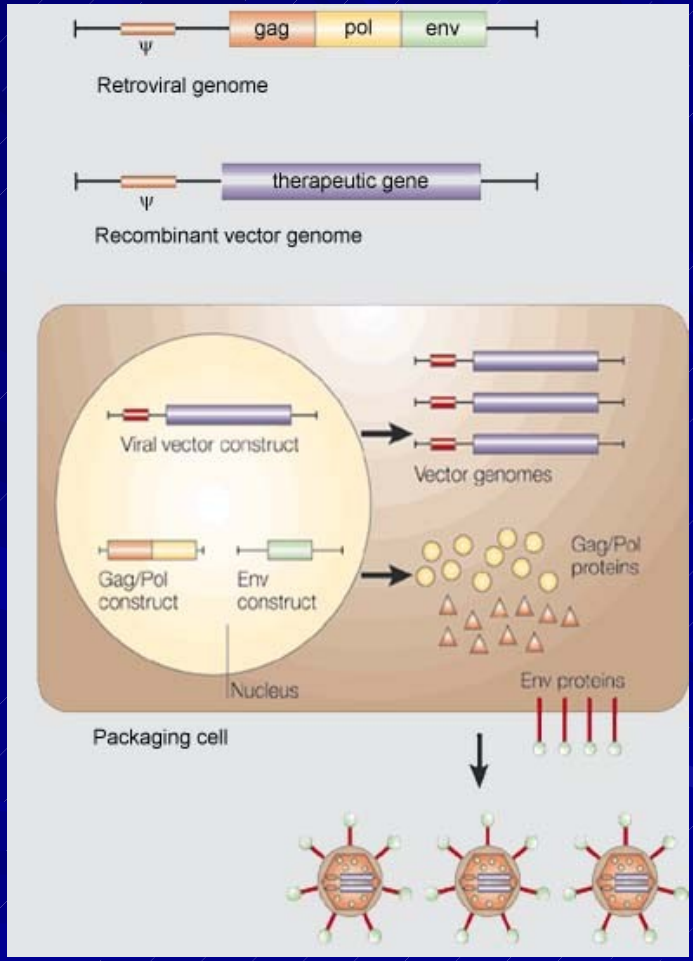
Methods of Delivery

- Gene Transfer Vector used to insert gene
- Viral Vectors have been most successful
- 2 types of attenuated viruses used
  - Replication-Competent: Can reproduce and spread from cell to cell in the human body
  - Replication-Defective: Naturally or Artificially cannot replicate, dies after first infection cycle



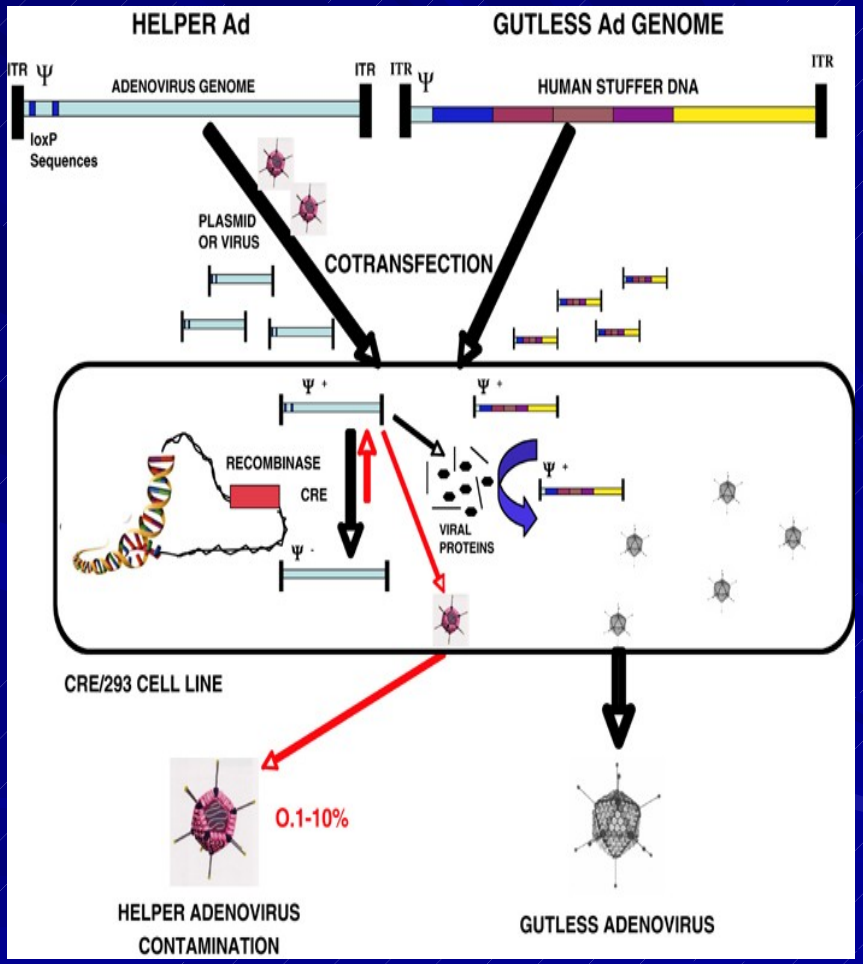
Retrovirus Vectors

- Replace gag, pol, and env genes in retrovirus, and package in a cell with these genes
- Problem: Integrase enzyme inserts gene anywhere in genome
  - Can cause cancer
  - Use zinc finger nucleases or control sequence to direct integration site
- Lentivirus (HIV, SIV, FIV)
  - Long incubation period
  - Can deliver large amounts of genetic information



Adenovirus Vectors

- Do not integrate DNA with genome, so less risk of mutations
- Can use up to 30kb of therapeutic gene
- Most commonly used are replication-deficient Subgroup C Stereotype 2 or 5 (Respiratory Tract Infection)
- Promising in cancer treatment
- ‘Gutless’ or last-generation Adenovirus lowers Immune response and decreases chance of viral expression.



Adeno-Associated Viruses

- AAV are non-pathogenic human single-strand DNA parvoviruses
- Need helper adenovirus to proliferate
- Insert DNA in specific location on chromosome 19
- Difficult to produce, and only 4.7kb long
- However, non-pathogenic, so there is no immune response to the virus

Limitations and Ethical Concerns

- Immunogenetic Responses
- Insertion Mutagenesis
- Toxicity
- Short-Lived Nature
- Uncontrolled virus replication or mutation
- What are Disabilities and Disorders, and should they be cured? (e.g. color-blindness, autism)

Non-Viral Vectors

- May be more effective:
  - Simple large scale production
  - Low safety risks
  - Low levels of transfection overcome
- Naked DNA
  - Intramuscular plasmid injection successful, but low transfection rate
- Oligonucleotides
  - used to limit expression of defective genes
- Lipoplexes and Polyplexes
  - Lipids or polymers used to surround plasmid, protecting it and increasing transfection efficiency

Gene Therapy in Oncology

- Immunopotentiation
  - Increase Immune Reaction to Tumor
- Oncogene Inactivation
  - Deactivated by Oligos that target Oncogene Promoter Region
- Restoration of Tumor Suppressor Gene
- molecular chemotherapy
  - Implant herpes simplex virus thymidine kinase (HSV/TK) into tumor, produces toxic waste

Developments in Gene Therapy

- May 2006 - Use of microRNA to limit transgene expression
- May 2008 – Gene (*RPE65*) successfully implanted into retina to cure blinding disease
  - http://content.nejm.org/cgi/content/full/NEJMoa0802315

Conclusions

- Gene Therapy can be used to cure or treat many genetic disorders
- Safety issues and effectiveness limitations cause the technology to not be able to fully solve genetic problems.

- Thank you…Questions?

Sources

- http://en.wikipedia.org/wiki/Gene_therapy
- http://www.microbiologybytes.com/virology/peel/p
- http://www.genetherapynet.com
- http://www.ornl.gov/sci/techresources/Human_Ge