Biology 311 Human Genetics Fall 2004

Lecture: Gene Therapy

Readings:

Mountain, A. (2000) Gene therapy: the first decade. TIBTECH 18, 119-128.

Wu, N. and Ataai, M. M. (2000) Production of viral vectors for gene therapy applications. Current opinion in biotechnology 11, 205-208.

Chapter 21 pp. 616-629

Presentation:

Presentation on gene therapy by Nicholas Simon Spring 2004, Biology of Cancer

Outline:

1. **Gene therapy strategies**
2. **Disease targets**
3. **Gene therapy vectors**

a. **viruses**
b. **non-viral**

4. **Future prospects**

Lecture:

1. Gene therapy strategies

Gene therapy= The treatment or prevention of disease by gene transfer.

Two major approaches:

a. Gene addition (supplementation): Introduction of a new copy of a gene to supplement an existing copy. Gene therapy protocols in clinical trials all use this strategy.

b. Gene replacement: Correction or replacement of a defective gene by a functioning gene. Technically much more difficult.

Other strategies are shown in Fig. 21.4

2. Disease targets

- Existing therapies are directed at somatic cells, not germ line (gamete) cells.
- Cancer cells are a major target; most gene therapies for cancer introduce versions of the tumor suppressor gene p53 in order to restore normal cell control.
- Some diseases caused by single gene defects (monogenic diseases) are targets for gene therapy. For example, cystic fibrosis.
- Some infectious diseases are being treated with gene therapy approaches.
- Some genetic diseases are not readily approachable with gene therapies yet due to the large size of the gene (muscular dystrophy) or inability of the vectors to direct the gene to the correct location.

Drug and therapeutic testing: Clinical trials

Phase I: Small trials of 10-15 individuals to determine toxicity of a treatment.

Phase II: Small trials of 10-50 individuals to determine toxicity, to test possible dosages or delivery methods, and to obtain preliminary results on effectiveness.

Phase III: Large trials of several hundred to a thousand individuals to determine whether a treatment strategy is safe and effective. A successful trial provides results that lead to approval of drugs or more widespread applications of new therapies.

3. Gene therapy vectors

a. viruses

Adenovirus:

- Human virus, causes respiratory tract illness
- Double stranded DNA virus of 36 kb
- Can be grown to high titer
- Can infect a variety of cell types
- Infection can trigger an adverse immune response
- Size limit of introduced gene about 7.5 kb
- Most successful vector so far for human gene therapies
- Most useful versions are gutted, removing viral genes that trigger the immune response, but require growth in presence of helper virus
- Short duration of expression

Adeno-associated virus (AAV):

- Human virus, not toxic to infected cells, doesn't trigger an immune response
- A single-stranded DNA virus with genome 4680 bases long

- Replication requires functions of a helper virus such as adenovirus or herpes simplex
- Can infect both dividing and non-dividing cells
- Can only carry about a 4.5 kb gene
- Low production yield
- New generation vectors engineer helper functions on other plasmids or viruses

Retroviral vectors:

- Most are based on a mouse leukemia virus (MMLV=Moloney murine leukemia virus)
- Only infect actively dividing cells; especially useful for tumor therapy
- Single stranded RNA virus, DNA copy integrates into host genome
- Size limit 8 kb
- Vector replication and packaging functions are provided by engineered cell line
- Problems: Recovery of replication competent retrovirus and low titer

Lentivirus (HIV) vectors:

- HIV causes lethal disease, safety concerns a disadvantage
- Single-stranded RNA virus, DNA copy integrates into host genome
- Infect proliferating and non-proliferating cells
- Can infect blood-forming (hematopoietic) stem cells
- Size limit of 8 kb

Herpes simplex virus:

- HSV causes many different diseases in humans, cytotoxic
- Double stranded DNA virus
- 150 kb viral genome with 80 viral genes
- Capacity as a vector is about 30 kb
- Can infect a wide range of cells, including neurons
- Does not insert its DNA into host genome

b. non-viral

- Low immunogenicity; safe
- Easy to manufacture and store
- Inefficient transfection and short duration of expression

Naked DNA

- Works well for muscle, heart, skin
- DNA vaccinations can induce immune response against encoded antigens
- Not suitable for targeted therapies
- New methods allow injection into tissues without needles: "gene gun" or Intraject