Tissue Specific Targeting of the Liver

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Why target the liver?

- Involved in many metabolic diseases
- Roles coupled with circulating blood
- Accessible to large molecules

Methods of Gene Transfer

- Non-Viral
  - Naked DNA
  - Liposomes
  - Molecular conjugates
- Viral
  - Retroviruses
  - Adenoviruses
  - Other viruses

Non-viral Transfection

- Advantages
  - Non-oncogenic
  - No limits on insert size
  - Can transfect non-dividing cells
- Disadvantages
  - Less efficient
  - Transient gene expression

Overcoming the Efficiency Barrier

Overcoming the Efficiency Barrier

- Naked DNA
  - Over 20 years ago, naked or complexed with calcium phosphate: low expression
  - 1996, J. Wolff able to express a marker gene in large fraction of liver cells
  - Clamp afferent and efferent liver vessels
- Liposomes
  - 1996, Expression is too low and too transient for clinical use

Asialoglycoprotein Receptor Targeting System

- DNA is coupled to polylysine which is coupled with asialoglycoprotein
- Excellent results *in vitro*
- *In vivo*, maintained specificity but low expression
- Many systems target asialoglycoprotein, but success in vivo has yet to be shown

Viral Vectors

- Retroviruses
- Adenoviruses
- Other Virus Vectors
  - Hepatitis Virus
  - Herpes simplex virus
  - Adenoassociated virus
- Lentivirus

Retroviruses

- Usually derived from Moloney murine leukemia virus (MMLV)
- Insert size of < 8 kB

Advantages

- Integrates into host genome
- Stable transfection of dividing cells

Disadvantages

- Transfects only dividing cells

Initiating Cell Cycle Progression

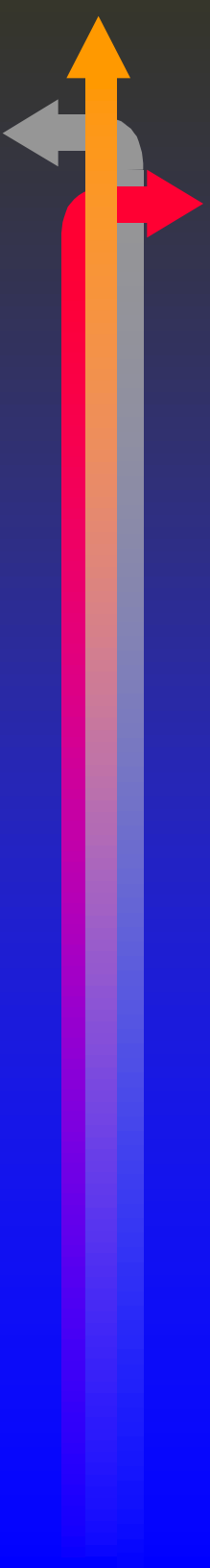
- Liver cells are arrested in G_o phase

- *Ex vivo* approach
  - Culturing liver cells in appropriate medium
  - Specificity of virus is irrelevant

- *In vivo* approach
  - Stimulating liver regeneration *in situ*
  - Specificity of virus is important

Ex vivo Approach

- Harvested by surgical biopsy
- Infected by retroviruses
- Reinjected into the liver
- Animal studies
  - Promising
  - Partial correction of type I tyrosinemia, familial hypercholesterolemia, and α_1-antitrypsin deficiency



Human studies

- 3 millions cells injected
- no convincing therapeutic effect in 5 patients tested
- one showed modest decrease of cholesterolemia
- most reinjected cells did not settle in liver

In vivo Approach

- Less work than *ex vivo* approach
- Induced by surgical hepatectomy, chemical injury, drugs
- Best when corrected cells have selective growth advantage

- Rodent Studies
  - Expression for periods longer than 1 year
  - Up to 68% transduction efficiency

In vivo Approach

- Large Mammal Studies
  - Poor transduction efficiency in dogs
  - Potentially difficult and dangerous in humans
- Increasing Specificity
  - Manipulating retroviral envelope to bind to specific receptor
  - Chemical attachment of lactose promotes binding to asialoglycogen receptor

Adenoviruses

- Insert size of < 7.5 kB
- Advantages
  - Transfects both dividing and non-dividing cells
  - Many vectors are specific to the liver
- Disadvantages
  - Triggers immune response
  - Transient expression

Defeating the Immune Response

- Immunosuppressive drugs
- Make immune system tolerant of adenoviral proteins
- Modifying vectors to decrease immune response

Hepatitis Viruses

- Work began in early 1990's using hepatitis B viruses to transfect liver cells
- Still no evidence of gene transfer and expression

Herpes Simplex Virus (HSV)

- Insert size < 20 kB
- Advantages
  - Large insert size
- Disadvantage
  - Neuron specificity
  - Transient expression
  - Potential of generating infectious HSV

Adenoassociated Viruses (AAV)

- Insert size < 4 kB
- Advantages
  - Stable Transfection
  - Site Specific Integration
- Disadvantages
  - Small Insert Size
  - Difficult to produce

Lentiviruses

- Derived from HIV
- Advantages
  - Stable Transfection
- Disadvantages
  - Difficult to produce