Tissue Specific Targeting of the Liver

Chung-Han Lee
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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 \textit{in vitro}
- \textit{In vivo}, maintained specificity but low expression
- Many systems target asialoglycoprotein, but success in \textit{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 G₀ 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 $\alpha_1$-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 transf ect 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