



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

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Methods of Gene Transfer

»» Non-Viral

- ❖ Naked DNA
- ❖ Liposomes
- ❖ Molecular conjugates

»» Viral

- ❖ Retroviruses
- ❖ Adenoviruses
- ❖ Other viruses

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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

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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

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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

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Viral Vectors

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

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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

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Initiating Cell Cycle Progression

- ❖ Liver cells are arrested in G_0 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

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Ex vivo Approach

- ❖ Harvested by surgical biopsy
- ❖ Infected by retroviruses
- ❖ Reinjecting 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

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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

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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

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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

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Herpes Simplex Virus (HSV)

- ❖ Insert size < 20 kB

- » Advantages

- ❖ Large insert size

- » Disadvantage

- ❖ Neuron specificity

- ❖ Transient expression

- ❖ Potential of generating infectious HSV

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Adenoassociated Viruses (AAV)

- ❖ Insert size < 4 kB

» Advantages

- ❖ Stable Transfection

- ❖ Site Specific Integration

» Disadvantages

- ❖ Small Insert Size

- ❖ Difficult to produce

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Lentiviruses

- ❖ Derived from HIV

- » Advantages

- ❖ Stable Transfection

- » Disadvantages

- ❖ Difficult to produce