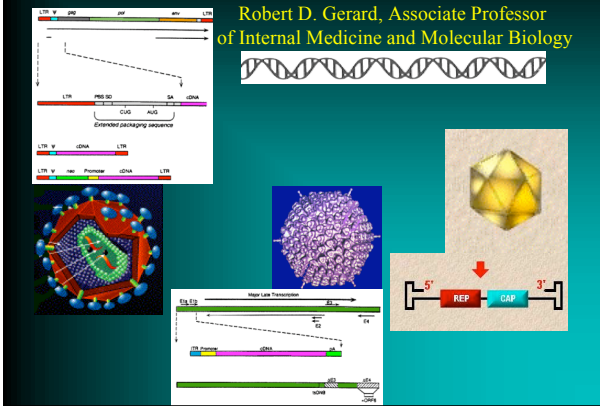


Experimental Approaches to Complex Diseases and Gene Therapy

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Viral Vectors

- Retrovirus (Lentivirus)
- Adenovirus
- Adeno-Associated Virus
- Herpesvirus, Vaccinia (poxvirus)
- Engineered Affinity Vectors (e.g. phage, RV, Ad)

Approaches to Gene Transfer/Gene Therapy

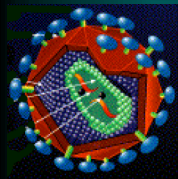
Target Dependent Variables

- What is the mechanism of gene action?
 - Systemic
 - Enzymatic
 - Endocrine
 - Paracrine
 - Autocrine
- What cells will be modified?
 - Vector choice
- What efficiency is required?
 - Vector choice
 - Method of delivery
- What is the required duration?
 - Vector
 - Promoter
- What pattern of expression is needed?
 - Temporary vs durable
 - Specific vs widespread

Approaches to Gene Therapy: Comparison of Viral Transduction Systems

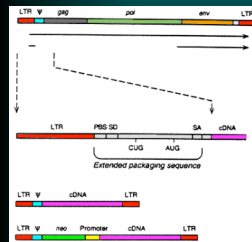
	RV	Lentivirus	Ad	guttled Ad	AAV
Insert Size	~ 8kB	~ 8kB	~ 8kB	>30Kb	~ 4kB
Titer: crude	10 ⁵ -10 ⁷	10 ⁷ -10 ¹⁰	10 ⁷ -10 ⁹	10 ⁷ -10 ⁹	10 ⁷ -10 ⁸
purified	10 ⁷ -10 ¹⁰	10 ⁷ -10 ¹⁰	10 ¹² -10 ¹³	10 ¹² -10 ¹³	10 ¹⁰ -10 ¹²
Integration	Yes	Yes	Rare	Rare	Rare
Expression	Variable	Extended	Transient	Extended	Extended
Delivery	Poor	High	Very high	Very high	Very high
Quiescent cells	No	Yes	Yes	Yes	Yes

Approaches to Gene Therapy: Viral Vectors: Retrovirus



- Enveloped ssRNA virus
- Cell attachment via virally-encoded env glycoprotein
- Receptor-mediated uptake
- Recombinants generated in cell lines that supply viral functions in *trans*
- Trophism is packaging line dependent
- Integrating vector- stable expression
- Proliferating cells required

Approaches to Gene Therapy: Viral Vectors: Retrovirus



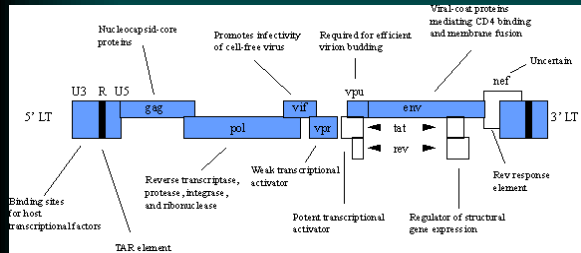
Advantages

- High transduction efficiency
- Insert size up to 8kB
- Integrates into host genome- sustained expression
- Extremely well-studied system
- Guttled vector- viral proteins not expressed in host

Disadvantages

- Requires dividing cells for infectivity
- Low titers (10⁶- 10⁷)
- Integration is random (insertional mutagenesis)
- In vivo delivery poor

Approaches to Gene Therapy: Lentivirus Vectors

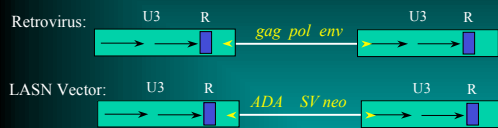


- Cell division unnecessary for infection/integration
- Efficiently infects cells in vivo

Gene Therapy of ADA(-)SCID

- Adenosine deaminase - deamination of adenosine to inosine
- Genetic deficiency - accumulation of dATP, T-cell cytotoxicity
- Severe Combined ImmunoDeficiency (SCID)
- Palliative therapy with PEG-ADA
 - partial correction of lymphopenia
 - partial restoration of immunoreactivity
- HLA identical BMT is curative

Gene Therapy of ADA(-)SCID



- Autologous T cells harvested, transduced, amplified
- No selection of transduced cells
- Gene transfer into hematopoietic stem cells - limited efficacy
- Retransfusion (repeated therapy over 2 years)
- Continued PEG-ADA therapy

Gene Therapy of Cystic Fibrosis

M+ - C 4 6 7 9 12

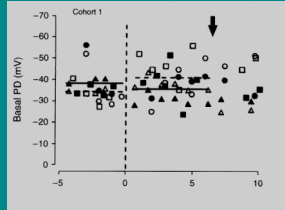


RT/PCR analysis

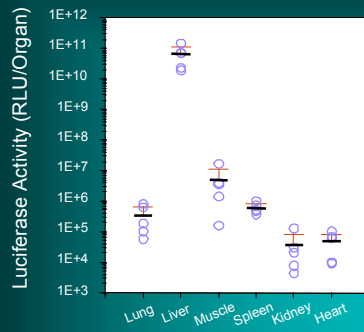
- Detectable gene expression
- Low efficiency of gene transfer
- Insignificant biological effect

Adenovirus-mediated transfer of a CFTR gene into nasal epithelium

Trans epithelial Potential Differences



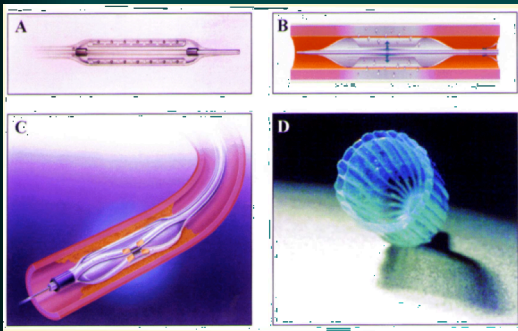
Gene Expression in Organs after Intravenous Injection in Mammals



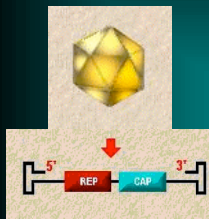
Gene Therapy of Cancer

- Carcinoma, sarcoma, melanoma, lymphoma
- >240 clinical trials
- Immunopotentialiation
 - Epitope expression (MHC-1, MART-1, HBsAg)
 - Co-stimulator expression (CD80/B7)
 - Cytokine expression (IL-2, IL-4, IL-12, GM-CSF, IFN)
- Cell-cycle control (p53, cdc-cdk, p21, dnRb)
- Signal transduction (K-ras, erb-B2)
- Apoptosis (antisense bcl-2, bax, bclxs)
- Targeted cytotoxicity (HSVtk-gancyclovir)
- Cancer vaccines (HPV)
- Replication competent virus (ONXY015)

Percutaneous Local Drug Delivery Devices

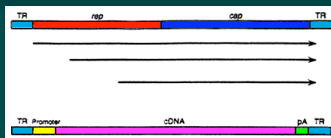


Approaches to Gene Therapy: Viral Vectors: Adeno-associated virus



- non-pathogenic human parvovirus
- ssDNA virus, + or - strand
- only two genes, rep- replication and cap- capsid proteins
- replication is dependent upon helper- Ad or HSV
- recombinants replace rep and cap
- broad tissue tropism
- persistent expression
- newer production strategies:
 - Replace helper virus with plasmid
 - Much higher titers ($>10^{10}$)
- readily purified for in vivo use

Approaches to Gene Therapy: Viral Vectors: Adeno-associated virus



- Recombinant AAV: gutted vector-no viral gene products
- Strict packaging limit ~ 4.7 kb
- Minimal immune response
- Host response to capsid does not preclude readministration
- WT virus integrates specifically at 19q13ter- recombinants integrate randomly

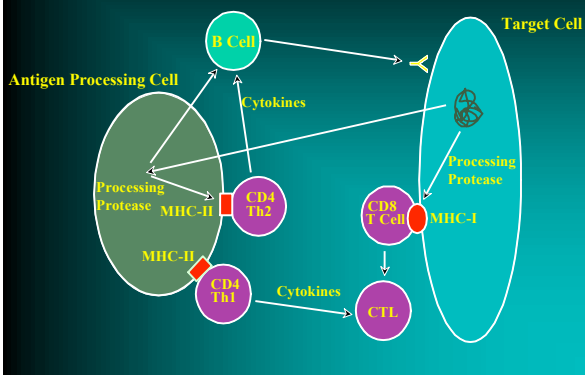
AAV Gene Therapy of Hemophilia B

- Hemophilia B due to clotting Factor IX deficiency
- Human factor IX AAV vector
- Phase I clinical trial
- IM transfer/liver gene transfer
 - secretion of Factor IX into bloodstream
- long-term expression of factor IX
- reduced clotting time

Problems in Human Gene Therapy

- Innate immunity to vector
- Pre-existing immunity to vector
- Immune responses to foreign gene, vector
- Limited efficiency
- Short duration of expression
- Lack of stem cell targeting
- Systemic/local toxicity due to transgene or virus
- Unforeseen complications-death

Immune Response to Transduced Gene Product



Immune Response to Transduced Gene Product

Effect of Host Immune Status on Duration of Gene Expression

Immune Status	Site	5d	21d	30d
SCID		100X	10,000X	1000X
MHC-1(-)		1X	1X	2-5X
MHC-2(-)		<5X	<5X	<5X
CsA		2-3X	2-3X	<2X

Websites of Interest

<http://www.asgt.org>
<http://www.esgt.org>

<http://www.peds.umn.edu/Centers/gene/>
<http://www.med.unc.edu/genether/>

<http://www.nih.gov/od/oba/>

<http://www.wiley.co.uk/genmed/clinical/>

<http://www.tulane.edu/%7Edmsander/garryfavweb.html>
