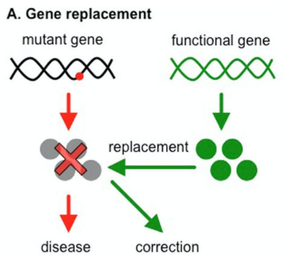
Gene Therapy: Mechanisms and Potential Disease Applications

Katie Green

BLU9A

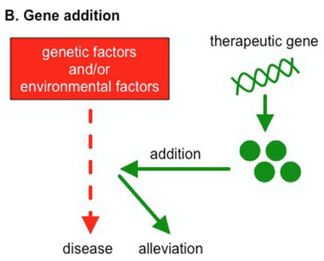
What is gene therapy?

The basic concept involves inserting a normal gene into a patient with a disease caused by a defective gene. Strategies include gene replacement and gene addition.



Gene replacement:

- Gene mutation represented by red dot, prevents appropriate protein production and leads to disease
- Insertion of functional gene copy allows for normal protein production to correct disease

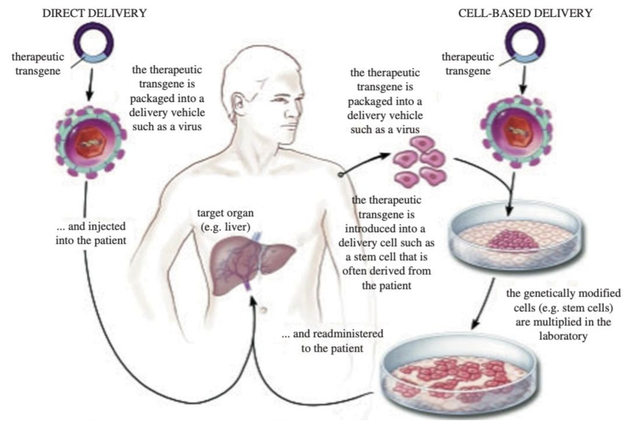


Gene addition:

- Disease is not necessarily caused by genetic mutation alone
  - Genetic and environmental factors can lead to disease via a complex cascade
- Therapeutic gene inserted to target a specific part of the disease cascade mechanism

Gene Delivery Methods

Two main methods for delivery of a normal gene: direct (in-vivo) delivery and cell-based (ex-vivo) delivery techniques.



FDA Approved Gene Therapy Treatments

The FDA has approved treatments to target melanoma tumors with a genetically modified herpes simplex viral vector, cancer cells with CAR-T cell vectors, inherited retinal disease and RPE65 mutations with AAV vectors, non-Hodgkin lymphoma with CAR-T cell vectors, and spinal muscular atrophy with an AAV vector delivering the SMN1 gene. Many other clinical trials are being carried out, however the results of gene therapy trials are not always as expected and may not provide desirable outcomes.

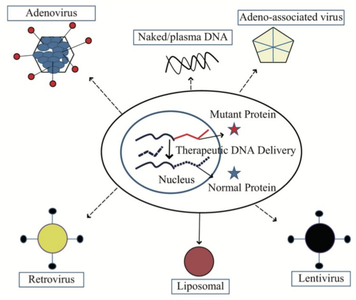
FDA-approved gene therapy products

Tradename	Manufacturer	Proper name	Function	Status	
Imlygic	Amgen Inc.	Talimogene laherparepvec	Genetically modified HSV immunostimulatory injected into melanoma tumors	FDA approved	2015
Kymriah	Novartis Pharmaceuticals Co.	Tisagenlecleucel	T cells are genetically engineered to express a chimeric cell surface receptor to target cancer cells	FDA approved	2017
Luxturna	Spark Therapeutics Inc.	Voretigene rseparvovec-rzyl	AAV vector-based gene therapy for patients with inherited retinal disease and RPE65 mutations	FDA approved	2017
Yescarta	Kite Pharma Inc.	Axicabtagene ciloleucel	CAR T therapy for adults living with certain types of non-Hodgkin lymphoma.	FDA approved	2017
Zolgensma	AveXis, Inc	Onasemnogene abeparvovec-xioi	AAV delivery of SMN1 gene for treatment of spinal muscular atrophy	FDA approved	2019

Vectors: Delivery Vehicles for Gene Insertion

Vectors serve as messengers that infect cells and integrate their genetic material into the host genome, and later use the cellular machinery to produce proteins that they encode.

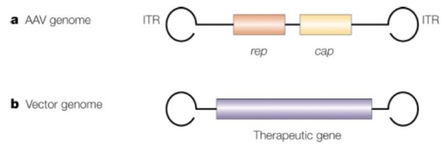
- Types of vectors
  - Viral
    - Retrovirus
    - Lentivirus
    - Adenovirus
    - Adeno-associated virus (AAV)
    - Recombinant AAV (rAAV)
    - Oncolytic viruses
  - Non-viral
    - Naked DNA/RNA
    - Plasmid DNA
    - Bacteria

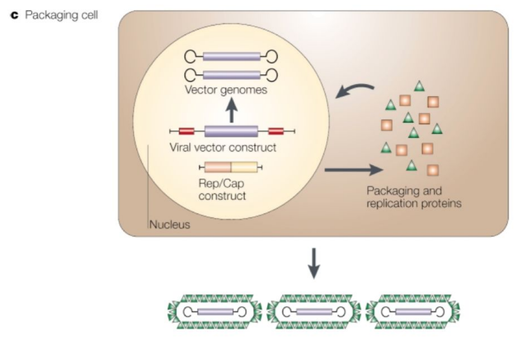


Engineering Vectors to Contain Therapeutic Gene of Interest

AAV vectors:

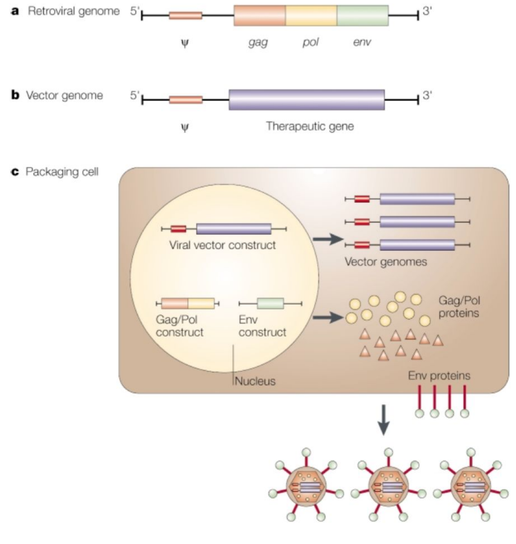
The AAV genome (a) contains essential sequences for transduction, which include the inverted terminal repeats (ITRs) and the rep and cap genes. Rep and cap are replaced in the vector genome (b) with the therapeutic gene. Rep and cap protein products provided by packaging constructs are essential for single-stranded DNA to be produced (c). These single-stranded DNA genomes are encapsulated by coat proteins. This non-enveloped virus is put together in the nucleus, and then some adenovirus helper proteins including E1A, E1B, E2A, and E4 assist in replication of the AAV.





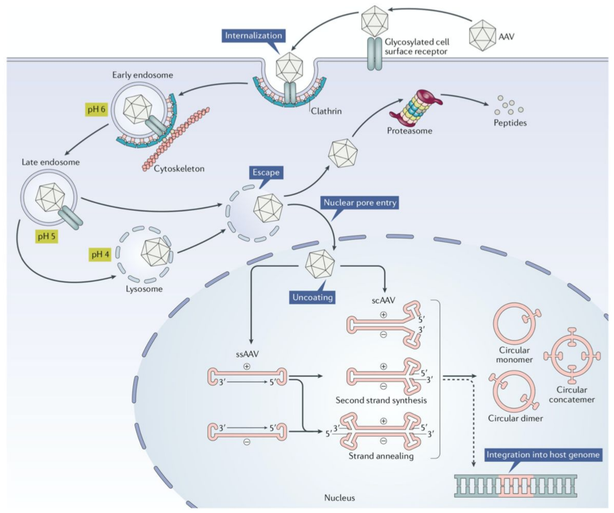
Retrovirus-based vectors:

Genes included in the retroviral genome include gag, pol, and env (a). The packaging sequence, Ψ, distinguishes viral RNA from the other RNA in the cell. The Ψ gene is additionally recognized by viral proteins for packaging. The vector genome (b) contains Ψ, as well as the therapeutic gene that replaces gag, pol, and env. To make normal viral replication unlikely, gag and pol are separated from env. Because of the Ψ gene, the vector genomes are encapsulated under the membrane with the pol and gag proteins. Once the virus leaves the packaging cell, it can then infect target cells (c).



Integration of Exogenous Genetic Material into Host via AAV Vector

AAVs engineered to contain a correct copy of a gene are recognized by host cell glycosylated cell surface receptors, triggering viral internalization by clathrin-mediated endocytosis. AAV moves through the cytosol in an endosome with the help of the cytoskeleton. After escape from the endosome, AAV is transported into the nucleus and uncoated. Alternatively, AAV may undergo proteolysis by a proteasome. Once uncoated in the nucleus, the genetic material is able to integrate into the host genome.



Disease Applications

In order to utilize gene therapy techniques to treat disease, certain conditions must be met. This contributes to the complexities of the technique.

Prerequisites:

- Identify disease and defective gene/factors responsible for disease
- Identify gene mutation
- Establish relation of mutation to disease pathophysiology
- Clone normal healthy gene
- Identify target cell/tissue/organ

Once the prerequisites are met, a vector must be chosen. Each vector has different characteristics, so they must be suited for the specific disease.

Vector	Tissue Tropism	Potential Therapeutic Disease Targets
AAV1	Skeletal muscle, lung, CNS, retina, pancreas	HIV, CMT1A
AAV2	Smooth muscle, skeletal muscle, CNS, liver, kidney	AADC deficiency, Parkinson's Disease
AAV3	Hepatocarcinoma, skeletal muscle, inner ear	
AAV4	CNS, retina	
AAV5	Skeletal muscle, CNS, liver, lung, retina	MPS-IIIB, Hemophilia A, Hemophilia B
AAV6	Skeletal muscle, heart, lung, bone marrow	Hemophilia A, Hemophilia B
AAV7	Skeletal muscle, retina, CNS,	
AAV8	Liver, skeletal muscle, CNS, retina, pancreas, heart	Achromatopsia, Hemophilia A, Crigler-Najjar Syndrome, Hemophilia B, HIV, Pompe Disease
AAV9	Liver, heart, brain, skeletal muscle, lungs, pancreas, kidney	Batten disease (CLN6), Spinal Muscular Atrophy, Giant Axonal Neuropathy, Duchenne Muscular Dystrophy
AAV10	Liver	
Lentivirus		Transfusion-dependent β-thalassemia, Sickle Cell Disease, Wiskott-Aldrich Syndrome, X-SCID (figure below, part a), ADA-SCID, melanoma (b), CD19-expressing B-cell malignancies (c)