Hemophilia and Gene Therapy

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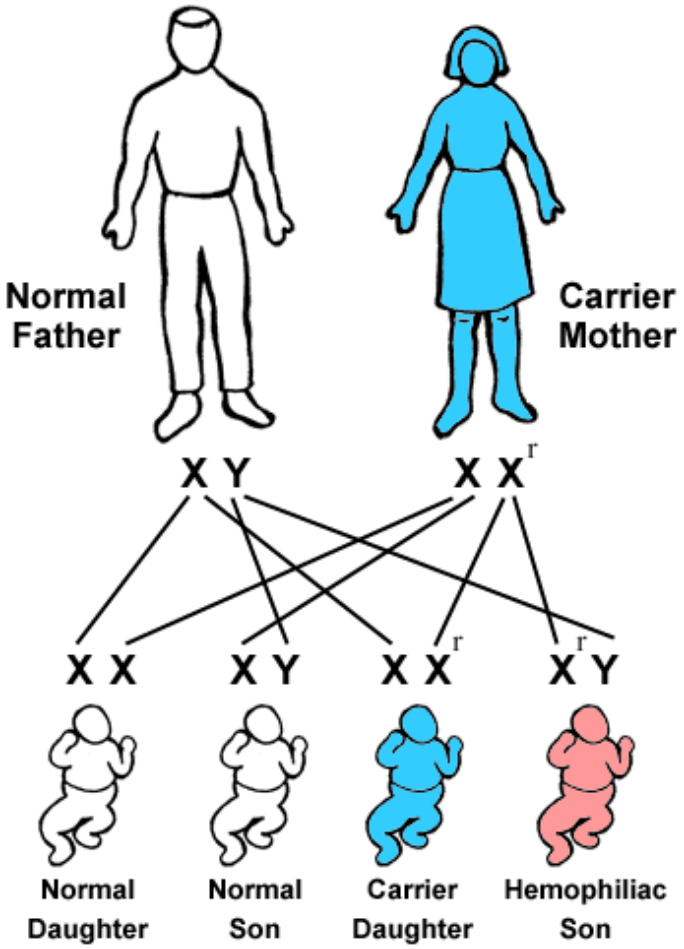
Overview

- Hemophilia, the disease
- Gene therapy
- Hemophilia as a target for gene therapy
- Gene delivery systems
- Clinical trials
- New methods
- Future of gene therapy for hemophilia

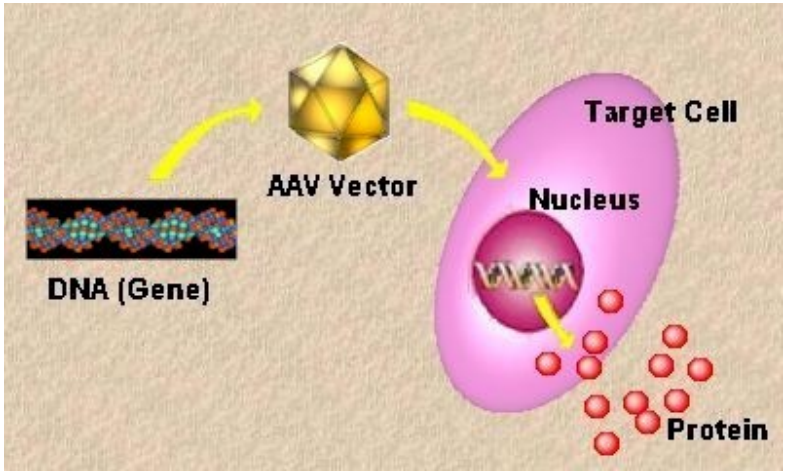
Hemophilia, the disease

- X-linked, recessive bleeding disorder
- Deficiency in activity of coagulation factor VIII (A) or factor IX (B)
- Hemorrhage, easy bruising, prolonged bleeding
- Carrier detection, prenatal diagnosis
- Prophylactic treatment with infusions of factor VIII or IX concentrates every 2-3 days



http://ez002.k12.sd.us

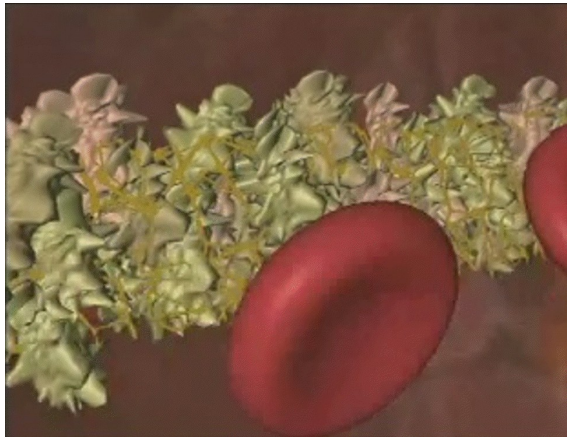
Gene therapy



- "Introduction of foreign genetic material into a cell with therapeutic intent." (Pasi 2001)
- Replacement of mutated factor VIII, IX genes with functional factor VIII, IX genes
- Desired result: Modified cells can produce functional protein

http://www.nettally.com/

Hemophilia as a target for gene therapy



- Single-gene disorder
- Genetic mutation → phenotype
- Tissue-specific expression and regulation aren't important
- Available small and large animal models
- Easy to assess efficacy in clinicial trials
- Even a small rise (1-2%) in coagulation factors can lead to significant therapeutic effects

http://www.mybloodyourblood.org

Gene delivery systems

Ex vivo

- Cells transduced in culture, then returned to patient
- Greater control over transfection conditions
- Unchanged cells can be removed and not transplanted
- But possibly difficult to transplant

In vivo

- Modification of cells within body
- Injection of vector with genetic material into body
- No transplantation issues
- More cost-effective
- But may provoke immune response
- Ultimate goal for gene therapy

Gene delivery systems

Retroviral factors

- Moloney murine leukemia virus (MoMLV)-based
- Can infect wide variety, integrate into host genome, relatively non-immunogenic
- Only in actively dividing cells
- Problems: insertional mutagenesis, inactivation by complement

Adenoviral vectors

- Relatively large, double-stranded DNA viruses
- Infect non-dividing cells
- Can transfer multiple copies of gene, *in vivo*
- Problems: Many humans may be immune to virus, not integrated into host genome
- 2nd generation: “gutless”

Gene delivery systems

Adeno-associated (AAV)

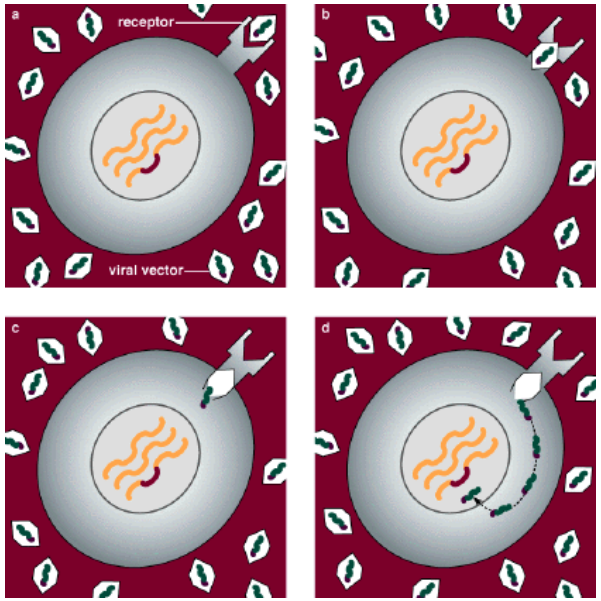
- Relatively small, single-stranded DNA parovirus
- Viral coding sequence replaced by transgene
- Non-dividing cells

Lentiviral vectors

- Retroviral vectors derived from lentiviruses (e.g.HIV-1)
- Integrate into genome, infect non-dividing cells
- Problems: limited range of cell targets, pathogens!
  - HIV – big risk of recombination → infectious virus
- Hybrid vector systems

Gene delivery systems

- Problems with viral gene transfer
  - Safety, immune responses, recombination events, cost and labor problems with production, germline transmission, insertional oncogenesis / tumors



Clinical trials

- Patient selection may vary between trials
  - Vector system, route of administration, target tissue, co-morbid states
- Heavily pretreated with factor concentrates, no inhibitory alloantibodies
- Since 2007, 5 phase I clinical trials

http://www.lifescipartners.net/

Clinical trials

- Hemophilia B
- Intramuscular injection of factor IX with rAAV (Kay et al. 2000, Mano et al. 2000, 2001)
- 8 patients enrolled
- No toxicity, no germ line transmission, no antibodies
- Expression of factor IX in muscle fibers, extracellular matrix 2 months later
- Modest increase in factor IX level in 2/6 subjects; reduction in infusions in 3/6

Clinical trials

- Hemophilia A
- *Ex vivo* (Roth et al. 2001)
- Cells transfected with factor VIII gene by electroporation
- 6 patients
- At 12 month followup, no serious toxicities or antibodies
- 4/6 with modest factor VIII activity levels, 2/4 with decreased bleeding frequency
- No improvement after 10 months

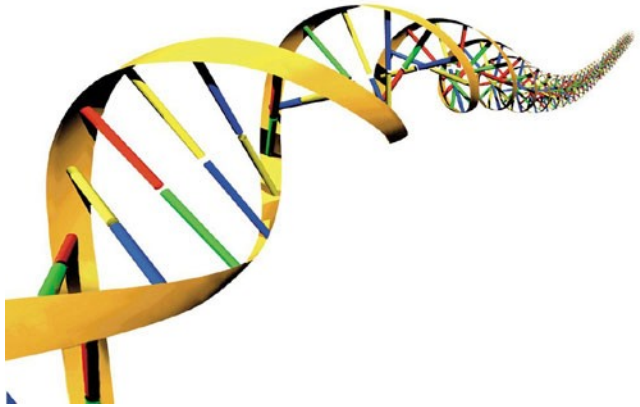
- Hemophilia A
- *In vivo* (Powell et al. 2001)
- MoMLV retroviral vector expressing factor VIII infused intravenously for 3 days
- 13 patients
- No adverse events or replication-competent retroviruses

New methods

- Novel gene delivery systems
- Direct injection of naked DNA
  - Problems: Low efficiency of transduction, not integrated into genome
  - Currently, gene expression in muscles and hepatocytes
- Transposons can stabilize chromosomal integration
  - Segments of DNA that can naturally move to different chromosomes; “Sleeping Beauty” encodes transposase to insert foreign genes into chromosomes (Ivics et al. 1997, Luo et al. 1998)
  - Naked DNA into mouse chromosomes → long-term factor IX expression in hemophilic mice (Yant et al. 2000)

New methods

- PTC124 can read through premature stop codons (L. Miller)
- Novel clotting factor, PEGylated liposomes (Spira 2006)
  - Longer bleeding-free period, reduce frequency of treatment



http://www.stern.de/

Future of gene therapy for hemophilia

- "Prevention" of hemophilia
  - Germline
- Treatment in developing countries
  - 80% of world without access to therapy for hemophilia
  - Current therapy too expensive and populations too large
  - Gene therapy as an alternative

http://www.wfh.org/

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