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
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 clinical trials
- Even a small rise (1-2%) in coagulation factors can lead to significant therapeutic effects

http://www.mybloodyourblood.org
## Gene delivery systems

<table>
<thead>
<tr>
<th><strong>Ex vivo</strong></th>
<th><strong>In vivo</strong></th>
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<tbody>
<tr>
<td>- Cells transduced in culture, then returned to patient</td>
<td>- Modification of cells within body</td>
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<tr>
<td>- Greater control over transfection conditions</td>
<td>- Injection of vector with genetic material into body</td>
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<td>- Unchanged cells can be removed and not transplanted</td>
<td>- No transplantation issues</td>
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<tr>
<td>- But possibly difficult to transplant</td>
<td>- More cost-effective</td>
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<tr>
<td></td>
<td>- But may provoke immune response</td>
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<td>- Ultimate goal for gene therapy</td>
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## Gene delivery systems

<table>
<thead>
<tr>
<th>Retroviral factors</th>
<th>Adenoviral vectors</th>
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<tbody>
<tr>
<td>▪ Moloney murine leukemia virus (MoMLV)-based</td>
<td>▪ Relatively large, double-stranded DNA viruses</td>
</tr>
<tr>
<td>▪ Can infect wide variety, integrate into host genome, relatively non-immunogenic</td>
<td>▪ Infect non-dividing cells</td>
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<td>▪ Only in actively dividing cells</td>
<td>▪ Can transfer multiple copies of gene, <em>in vivo</em></td>
</tr>
<tr>
<td>▪ Problems: insertional mutagenesis, inactivation by complement</td>
<td>▪ Problems: Many humans may be immune to virus, not integrated into host genome</td>
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<td></td>
<td>▪ 2nd generation: “gutless”</td>
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</table>
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

http://cmbi.bjmu.edu.cn
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
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/
Bibliography


