Principles and perspectives of gene therapy

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Older versions of lectures can be downloaded from the web page – Department of Medical Biotechnology at http://biotka.mol.uj.edu.pl/zbm/
Conditions for positive outcome...

1. Learning and understanding the information delivered during the lectures

2. Asking the questions during and after the lecture!

3. Supplementary materials:
   a) articles distributed during the course
   b) „Gene transfer to animal cells” – several copies are in the library

For Polish students:
   b) „Terapia genowa” red. Stanisław Szala (PWN, 2003)
   c) „Biotechnologia” – No 3/2007 (several copies are available in the library)

4. Passing the final exam …

   Test – multiple choice – questions will concern the information provided at the slides and those which will be explained in more details during the lectures – hence attending them is reasonable
Gene Transfer to Animal Cells

R.M. Twyman

ADVANCED METHODS
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Department of Medical Biotechnology was established as a separate unit in June 2005. Our main field of research is vascular biology, with particular interest in molecular mechanisms of angiogenesis, vasculogenesis, inflammation and oxidative stress. We are also interested in tumor cell biology. In our lab we investigate gene and cell therapy approaches to treat vascular disorders.
Parallel course

Gene transfer techniques in vitro

*seminars and practical course*

(Viral vectors in medical biotechnology)

Prof. Alicja Józkowicz – email: alicja.jozkowicz@uj.edu.pl

Magdalena Kozakowska – email: m.kozakowska@uj.edu.pl
What is gene therapy?

Application of nucleic acids for treatment of diseases

Gene-based therapeutics is broadly defined as the introduction, using a vector, of nucleic acids into cells with the intention of altering gene expression to prevent, halt or reverse a pathological process.

M. Kay, Nature Reviews Genetics, 2011
Gene therapy

Therapeutic gene (transgene) → Vector → Patient → Expression of therapeutic gene → Protein
Which diseases could be cured with gene therapy?

Is gene therapy necessary?
Gene therapy was born in... 1962

HPRT\(^{-/-}\) cells

HAT medium

HPRT\(^{+/+}\) cells

Prof. Waclaw Szybalski
McArdle Laboratory for Cancer Research, Wisconsin, Madison, USA
De novo and salvage pathways for nucleotide synthesis

De novo pathways:
- PRPP (5-Phosphoribosyl-1-pyrophosphate)
  - Blocked by antifolates
  - CHO from tetrahydrofolate

Salvage pathways:
- HGPRT (hypoxanthine-guanine phosphoribosyl transferase)
- APRT (adenine phosphoribosyl transferase)
- TK (thymidine kinase)
HAT medium

A selection medium for hybrid cell lines; contains hypoxanthine; aminopterin; thymidine. Only cell lines expressing both hypoxanthine phosphoribosyl transferase (HPRT+) and thymidine kinase (TK+) can survive in this medium. Aminopterin inhibits de novo synthesis of nucleosides, while HPRT and TK supply them from hypoxanthine and thymidine.
HAT medium is used to select hybridoma cells producing monoclonal antibodies.
Inborn error of metabolism – deficiency of HPRT

Lesch-Nyhan syndrome

HPRT

Guanine

Hypoxanthine

Xanthine

Urate

allopurinol

GMP

IMP

AMP

NH₄⁺
What is Lesch-Nyhan Syndrome?

Lesch-Nyhan syndrome (LNS) is a rare, inherited disorder caused by a deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT). LNS is an X-linked recessive disease— the gene is carried by the mother and passed on to her son. The lack of HPRT causes a build-up of uric acid in all body fluids, and leads to symptoms such as severe gout, poor muscle control, and moderate retardation, which appear in the first year of life. A striking feature of LNS is self-mutilating behaviors – characterized by lip and finger biting – that begin in the second year of life. Abnormally high uric acid levels can cause sodium urate crystals to form in the joints, kidneys, central nervous system, and other tissues of the body, leading to gout-like swelling in the joints and severe kidney problems. Neurological symptoms include facial grimacing, involuntary writhing, and repetitive movements of the arms and legs similar to those seen in Huntington’s disease. Because a lack of HPRT causes the body to poorly utilize vitamin B12, some boys may develop a rare disorder called megaloblastic anemia.

Is there any treatment?

Treatment for LNS is symptomatic. Gout can be treated with allopurinol to control excessive amounts of uric acid. Kidney stones may be treated by breaking up kidney stones using shock waves or laser beams. There is no standard treatment for the neurological symptoms of LNS. Some may be relieved with the drugs carbidopa/levodopa, diazepam, phenobarbital, or haloperidol.

What is the prognosis?

The prognosis for individuals with LNS is poor. Death is usually due to renal failure in the first or second decade of life.
Children suffering from deficiency of HPRT-Lesh-Nyhan syndrome
Children suffering from deficiency of HPRT- Lesh-Nyhan syndrome
Development of gene therapy

- Mechanisms of diseases: genes are known

- Tools: vectors
Main problems to solve in gene therapy

1. Efficient delivery of therapeutic gene

2. Safe delivery...

All is about vectors...
The fours barriers of successful gene therapy

Kay M, Nature Rev Genetics, 2011
vector

nucleic acid, which is used to deliver the therapeutic gene/therapeutic nucleic acid

Vehicle

A chemical substance, which improves delivery of nucleic acid to the cells
Genetic syringes - vectors

- Retroviral vectors
- Adenoviral vectors
- AAV vectors (adeno-associated)
- Plasmid DNA
Vectors

Carriers of the therapeutic nucleic acids
Retroviral expression system

Gag – core proteins, matrix, nucleocapsid
Pol – reverse transcriptase and integrase
Env – envelope glycoproteins
Retroviral vectors

- **gag** – structural proteins
- **pol** – reverse transcriptase
- **env** – envelope proteins

- long-term expression due to integration into cellular genome
First controlled trial of gene therapy - 1991

ADA deficiency – results in severe immunodeficiency syndrome
Gene therapy of ADA deficiency

Cloned gene

Cells removed

ADA

Gene transfer

Patient cells

ADA

Some cells now ADA

Select cells

Amplify

ADA cells

Return genetically modified cells to patient
First clinical trial of gene therapy - 1991

Retroviral vector containing correct ADA gene (cDNA) has been transduced into blood lymphocytes

This first clinical trial was not „pure” from the methodological point of view.

The patients have been treated concomitantly with enzyme injections – ADA-PEG.

Nevertheless, the marker transgene (neo) could be detected in the blood cells of the patients even more than 5 years after injection of modified cells.
Successful gene therapy
David Vetter - „Bubble Boy”

David has spent 12 years in a foil-protected environment. Finally has received the bone marrow transplantation from his sister, but unfortunately died due to Epstein-Barr virus infection.
X-SCID deficiency
X-linked severe combined immunodeficiency (X-SCID)

Lack of γc gene

Restoration of B and T lymphocytes and NK cells

Cytokines receptors

Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease

Science 2000: 28 April: 288: 669-672
Gene therapy is efficient in treatment of X-SCID

Ex Vivo Transduced CD34+ Cells
Expressing GammaC-R for X-SCID

Cord Blood → CD34+ Selection → CD34+ transduction
Fibronectin coated flasks → CD34+ expressing gamma-c receptor

Growth factors
SCF, Flt-3, IL-3, PEG-MDF

Murine Retroviral Vector
Gene therapy is efficient in treatment of X-SCID

Stem cells without correct γc gene

Gene therapy

Retroviral vector with a correct γc gene
Combining stem cells and gene therapy

*Future for treatment of some diseases?*
Gene therapy is successful in the treatment of diseases.

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<th>Disease type</th>
<th>Patients benefiting</th>
<th>First publication</th>
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<td>Immunodeficiency</td>
<td>17/20</td>
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<td>ADA-SCID</td>
<td>Immunodeficiency</td>
<td>26/37</td>
<td>2002</td>
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<td>Adrenoleukodystrophy</td>
<td>Neurologic</td>
<td>2/4*</td>
<td>2009</td>
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<td>Leber's congenital amaurosis</td>
<td>Blindness</td>
<td>28/30</td>
<td>2008</td>
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<td>Wiskott-Aldrich syndrome</td>
<td>Immunodeficiency</td>
<td>8/10</td>
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<td>Hemoglobinopathy</td>
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<tr>
<td>Hemophilia</td>
<td>Coagulation</td>
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<td>2011?</td>
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*Includes a patient treated too recently to see benefit.

Science, 7th October 2011