Problems in Gene Therapy and Transgenesis

Prof. Dr. Oliver Kayser
Technische Biochemie
TU Dortmund
# Producing transgenic animals

<table>
<thead>
<tr>
<th>Purpose:</th>
<th>Production of heterolog proteins in transgenic animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principle:</td>
<td>Integration of heterolog DNA with selected genetic information in fertilised eggs</td>
</tr>
</tbody>
</table>

## Methods:

<table>
<thead>
<tr>
<th>Method</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mikroinjektion</td>
<td>(-)</td>
</tr>
<tr>
<td>Retrovirus vectors</td>
<td>(-)</td>
</tr>
<tr>
<td>Genetically modified embryonic stem cells</td>
<td>(+++)</td>
</tr>
</tbody>
</table>
Finn Dorset

Remove mammary cells from udder

Mammary cells in culture

Mammary cell with nucleus

Electro-fusion

'Ovum (egg)

Enucleate ovum

Reconstructed cell with Scottish Blackface cytoplasm and Finn Dorset nucleus

Recover early embryo

Early embryo

Implant embryo in surrogate mother ewe's womb

"Dolly" - a Finn Dorset ewe born

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Dolly
Telomers
Advantage of sheeps and goats as transgenic animals

- high milk production capacity (2-3 l/day)
- short gestation period and quick maturation
- low capital investment
- easy handling and breeding
- high expression level of proteins (35g protein/1 l milk)
- easy downstream processing
- ongoing supply of product is guaranteed by breeding
Drug safety

- Genetic stability from animal to animal
- Variation in produktion and purity during lactation
- Microbial, varial, and mykoplasma contamination
- TSE, Scrapie
- Age and general conditions of animals
Atryn

• First recombinant therapeutic protein approved in 2009
• Anticoagulant for treatment of antithrombin III (AT alpha) deficiency in newborn
• 1 goat replaces 90,000 donators
Blood Clotting
Transgenic animals as disease model

- Knock out animals with directed genetic defect
- Testing of drugs and dosage forms
  - Hypercholesterinemia
  - Cancer
  - Coronar diseases
- Onco-Mouse as first example
Transgenic animals

Ageing-Mouse, Defect in DNA-Helicase
Transgenic salmon

• „Turbo-salmon“ AquAdvantage, AquaBounty Technologies

• Cloning of growth gene for improved growth (growth factors)

• Fish grows in 16 to 18 months instead of 3 years

• Triploid and not diploid (Biosafety)

http://www.nature.com/news/transgenic-salmon-nears-approval-1.12903
Kuru-Region in Papua-Neuguinea
Prions

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Bild 5: Das Scapie-PrP – oder genauer, dessen Konformation – phänomen sich in den Nervenzellen des Gehirns augenscheinlich durch eine Art Schachbrett-Konzeption (b) mit einem normalem Pendant (blau) in Kontakt tritt (a) und es dabei auf unbekannte Weise dazu bringt, sich ebenfalls in die Scapie-Konformation umzuformen (b). Dann beginnen beide zwei normale PrP-Moleküle an (c), die nach Umwandlung ihrerseits weitere normale Moleküle in die Scapie-Form bringen und so fort (durchlässiger Pfeil), bis die Menge an Scapie-PrP eine für die Zelle kritische Ausmaß erreicht hat (d).
Brain autopsy
Somatic Gene Therapy
# Defective Genes

<table>
<thead>
<tr>
<th>Disease</th>
<th>Genetic defect</th>
</tr>
</thead>
<tbody>
<tr>
<td>hemophilia A</td>
<td>absence of clotting factor VIII</td>
</tr>
<tr>
<td>cystic fibrosis</td>
<td>defective chloride channel protein</td>
</tr>
<tr>
<td>muscular dystrophy</td>
<td>defective muscle protein (dystrophin)</td>
</tr>
<tr>
<td>sickle-cell disease</td>
<td>defective beta globin</td>
</tr>
<tr>
<td>hemophilia B</td>
<td>absence of clotting factor IX</td>
</tr>
<tr>
<td>severe combined immunodeficiency (SCID)</td>
<td>any one of several genes fail to make a protein essential for T and B cell function</td>
</tr>
</tbody>
</table>
ADA und purine metabolism

\[
\text{IMP} \leftrightarrow \text{AMP} \leftrightarrow \text{ADP} \leftrightarrow \text{ATP} \quad \text{dAMP} \leftrightarrow \text{dADP} \leftrightarrow \text{dATP}
\]

Adenosin → Adenosine-Deaminase → Inosin → Acumulation

2-Deoxyadenosin → 2-Deoxyinosin
Basics of ADA-Deficiency

\[ \text{dAMP} \leftrightarrow \text{dADP} \leftrightarrow \text{dATP} \]

- Acumulation
- Apoptosis
- Inhibition of Ribonucleotid-Reductase
  - Consequence: No DNA-Replication
- Inhibition of T cell activation

Adenosin-Deaminase

2-Deoxyadenosin

2-Deoxyinosin

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EX VIVO vs IN VIVO

Tumor cells
fibroblasts
Hemopathic cells
Gene transfer in culture

Delivery to target
local
systemic

Vector

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Examples for local gene expression

Gene Therapy Vector

Double-Strand DNA
Adenovirus
CFTR

Targeted Cell
Host Chromosomes
Mutated CFTR
Airway Epithelial Cell

Intended Outcome
Cl–
CFTR Protein

Illustration: Seward Hung

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Vectors in gene technology

Biochemical vectors/Methods:
• viral vectors (Retro-, Adenovirus, AAV)
• ligands with polylysine
• 'Transferinfection'

Physical methods:
• Hydrodynamic pressure
• 'Biolistic Bombardment'
• electroporation
• direct tissue inclusion

Chemical vectors:
• cationic Liposomes
• polycations like DEAE-Dextran, Polyethylen-Imin-poly-Lysin-polymer
Retroviral vector system

Illustration: Seward Hung
Father of gene therapy participant says researchers acted 'irresponsibly'

February 2, 2000
Web posted at: 3:42 p.m. EST (2042 GMT)

From Medical Correspondent Elizabeth Cohen

(CNN) -- The father of a young man who died during a gene therapy trial at the University of Pennsylvania told a Senate hearing on Wednesday that researchers acted "irresponsibly" and they downplayed possible risks to his son.
Non-viral delivery

• The non-viral approaches provide a safety (no virus) to gene therapy. However, the route to nucleus for stable expression is not completely understood.
Vectors in gene technology

Biochemical vectors/Methods:
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- ligands with polylysine
- 'Transferinfection'

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Chemical vectors:
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Electroporation

Physical Principle

CHO-cells, 20 ms, 1 kV cm\(^{-1}\)
Electroporation

Examples

K562 cells with phGFPS65T transfection

Pros and Cons

+ High safety
+ High compliance
+ No risk of infection

- low transfection
- no tissue specificity
- transient expression

Target cells / organs
• liver
• skin
• muscle

Mainly:
Transdermal applications

Beta-Galactosidase transfection in vivo in muscle cells

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Mir et al. (1999) Proc Natl Acad Sci USA 96:4262
Biobalistics

**Principle**

- **Gene Gun**
- **Gold partikel**
- **Skin**

**Expression**

1-3 µm

**Examples**

- Application of vaccines
  - *Arilvax®, Hepagene®*
  - Powderject Inc: *Accell®*
  - Biorad Inc: *Helios®*
  - Transdermal Application

**Pros and Cons**

+ easy to use
+ using already existing technology

- high costs
- not only transdermal Application
- low efficacy
Powderject Inc.: Accell®
Liposomes

- This technique relies upon the negative charge of DNA-phosphate, cationic lipids, and cell surfaces (negative charge, sialic acid).
- Two classes of cationic lipids have been used:
  - two alkyl chain in each cationic lipid molecules (DOTMA)
  - cholesterol as a backbone.
- Dioleoylphosphoatidylethanolamine, a helper lipid is usually included to improve efficiency.
- Expression is transient for several days only.
Complexing DNA (+/- -ratio)

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DNA Condensation
Cellular uptake and migration

pH 4.5-6
Long persistence
DNA Hydrolysis

DNA degradation by nucleases
active uptake into nucleus
Polyethylenimine (PEI)

Gene transfer complex

- High stability
- Half life time: 30-60 min
- High toxicity (40-100 µg in mice)
- 50-100 nm
- Linear structure → high transfection
- High branching → low transfection
- Loading up to 800 kB

Uptake

- Coupling with ligands (Transferrin, Monoclonal antibodies, sugar, folate)

Migration / Transfection

- High stability
- "Sponge effect"
- transient expression

Nuclease degradation

Proton sponge

Inner tertiary amines neutralise low pH in endosome → swelling → breaking

Endocytosis

PEI-Gene transfer complex

Nucleus

H⁺

Cl⁻

H₂O

Behr (1997) Chimia 51:34

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Summary

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