Gene therapy

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Definition

Gene therapy is a medical process where the wildtype (normal) version of a gene is introduced into a patient's cells to treat the disease caused by the mutant form of the gene, which failed to function properly.

Types of gene therapy:

- ***In vivo*** gene therapy
- ***Ex vivo*** gene therapy

Vectors

Vectors are vehicles that can accurately target the therapeutic gene into the correct cells in the patient's body.

- viral or nonviral

Adenoviral vectors

- linear ds DNA
- non-integrating (time-limited expression)
- capacity: 8-36kb
- infect replicating and differentiated cells
- immunogenic
- the vehicle of choice for *in situ* gene therapy of solid tumors: shortlived presence of transduced cells is compatible with cancer gene therapy (expression of factor that eliminates cancer cells)

Retroviral vectors

- High Transduction Effciency
- Insert Size up to 8kB
- Integrates into host genome resulting in sustained expression of vector
- Vector proteins not expressed in host
- Requires dividing cells for infectivity (cannot pass through the nuclear membrane)
    - Solution: lentiviruses
- Integration is random
    - LTR = strong promoter
    - Can activate protooncogenes
    - Solution: SIN vectors
    - Lentivirus (intron integration) vs gamma-retrowirus (promoters, enhacers)
- In vivo delivery remains poor. Effective only when infecting helper cell lines

AAV vectors

- adeno-associated virus
- very small capacity (4 – 4.5kb)
- defective replication
- 12 serotypes, showing tissue-specific tropism
- no pathogenicity; low immunogenicity
- Transgene expression – sustained for ~2 years
- ineffective, nonspecific genome integration without Rep protein (most of vector DNA resides as episomes)

Liposomes

- hollow spheres surrounded with membranes made up of phospholipids
- can be used to transport therapeutic gene

Gendicine

- p53 protein
  - Transciption factor, responsible for cel life cycle regulation, DNA repair and induction of apoptosis
  - p53 mutation is the most frequent change observed in neoplasms
- Adenoviral vector carrying a functional copy of p53 gene

Table 3. Comparison of Protocol Efficiency

Protocol	N	4 Wks Treatment CR	PR	SD	PD	N	8 Wks Treatment CR	PR	SD	PD	N	4 Wks Follow-Up CR	PR	SD	PD
GTRT	63	8%	65%	27%	0%	62	45%	47%	8%	0%	56	64%	29%	7%	0%
RT	72	0%	40%	57%	3%	71	8%	62%	28%	0%	63	19%	60%	21%	0%

Comparison of GTRT group with RT group, p <0.01 in each comparison
CR: complete response, PR: partial response, SD: stabilized disease PD: progressive disease

- Available in China since 2003; treatment cost: 3800 yuan (~540$) / dose

Oncorine

- An experimental **oncolytic virus** (modified adenovirus)
- Adenovirus without E1B gene ➔ unable to inactivate p53 protein
  - Can replicate effectively only in cells with nonfunctional p53 (cancer cells)
- Clinical trials: greater response rate, lesser probability of progression in patients where Oncorine was introduced to the therapy
- Available in China since 2005; treatment cost: 3680 yuan (~520$) / dose

Examples of gene therapy

SCID

- severe combined immunodeficiency disease
- disease variant caused by mutation in **adenosine deaminase (ADA)**

    - **ADA deficiency** leads to accumulation of **toxic levels** of intracellular **purine metabolites** and thus impairment of T- and B-cells
    - loss of T- and B-cells functions presents clinically as **inability to recover from even a mild course of infection**
- ex vivo treatment
    - Lymphocyte cells were removed from patients and treated with retroviral vectors **carrying wildtype ADA gene**
    - modified lymphocytes were then returned to the patient's body
- Improved procedure:
    - hematopoietic stem cells instead of lymphocytes – isolated and transduced with retroviral vectors
    - before returning the cells - low-intensity, nonmyeloablative conditioning regimen (busulfan 2 mg per kg per day), to provide an initial developmental advantage to transduced HSCs and create space in the bone marrow
- Direct result of clinical trials: Strimvelis
    - gene therapy for SCID (ADA deficiency) available in Europe since May 2016
    - $648 000

B-ALL

- Acute B Lymphoblastic Leukemia
- Development of large numbers of immature lymphocytes
- The most common leukemia found in children
- CAR: Chimeric Antigen Receptor
    - Recognizes CD19 antigen, present on B-lymphocytes surface
    - Treatment of B-cell precursor acute lymphoblastic leukemia
- T-lymphocytes – isolated from patient, transduced with vectors carrying CAR
    - can recognize and destroy B-cells
- 30.08.2017: KYMRIAH (tisagenlecleucel)
    - gene therapy registered by FDA
    - $475000

Spinal muscular atrophy

- Genetic cause
    - SMN1 (survival of motor neuron 1)
        - 98% cases: exon 7 or 7 and 8 deletions
        - Phenotypic variability – depends on SMN2 polymorphism
- AVXS-101
    - AAV vector with functional copy of SMN1
    - 15 patients with SMN1 exon 7 deletion and two copies of SMN2 confirmed
        - 2 cohorts (high and low dose, 12 vs 3 patients); vector administerred iv
- Results
    - 100% survival at 20 months
    - Significant improvement of patients treated with high-dose vector

        - 11 sit unassisted and has the ability to feed orally
        - 2 walk unassisted
- May 2019
    - gene therapy registered by FDA: Zolgensma (onasemnogene abeparvovec); 2,1 mln $

β-thalassemia

- A hemolytic anemia disease
- Autosomal recessive
- Disruption of β-globin chain biosynthesis
- 22 patients aged 12-35, all requirring transfusion
- LentiGlobin BB305
    - VSV-G pseudotyped lentiviral vector
    - HbA^{T87Q} gene, encoding β-globin with T87Q substitution (inhibition of HbS polymerization)
- *ex vivo* treatment
    - CD34+ cells isolated from patient
    - Therapeutic gene carried by lentiviral vector
    - Engrafting modified cells after conditioning regimen (busulphan)
- Results
    - assessment of patients' condition 15-42 months after treatment
    - 15 patients - no longer needed transfusions
    - Other patients – annual transfusion volume reduced by ¾
- 29.05.2019r.: therapy registered by EMA
    - Zynteglo: 1.8 mln $

Leber congenital amaurosis

- Caused by mutations in RPE65
    - protein biosynthesized in retinal epithelium
    - Crucial for vision proces
        - Responsible for regeneration of 11-cis retinal
- AAV vector with wildtype RPE65
    - Administered directly to the retina
- Animal studies (dogs) delivered promising results
    - Behavioral tests resulted in normal vision in daylight
    - Dogs were able to navigate succesfule at dusk

- No positive changes obserced in the control group

- Clinical trials
  - Cideciyan et al. 2009, Jacobson et al. 2012, Bennett et al. 2016
  - A total of 29 patients, aged 11-46
  - Wildtype RPE65 in AAV vector
  - The results:
    - No severe ADRs
    - Significantly increased sensitivity to light
- 19.12.2017: registered by FDA
  - Luxturna; 850 000 $
  - available in Europe since November 2018

Cystic fibrosis

- Occurs when a person inherits two mutant **cystic fibrosis transmembrane conductance regulator** (CFTR) alleles.
- The normal CFTR is a large membrane protein that **regulates the concentrations of salt ions** in the cells (by moving them outside the cell)
- People with cystic fibrosis disease can make only the defective mutant cftr proteins in their cells, causing chloride ions to build up in the cells and sticky mucus outside of the cell
- More than 1,000 different mutations of the CFTR gene have been identified (different effects on the function of the CFTR protein); the most common in CF patients: 508Phe deletion (misfolded protein; 70% cases of CF)
- Ivacaftor (approved by FDA in 2012):
  - Only for patients >6 years old with specific CFTR mutations (G551D, G1244E, G1349D, G178R, G551S, S1251N, S1255P, S549N or S549R; ~3% of CF cases), resulting in a membrane protein with a constantly locked ion channel
  - The drug enables the opening of the ion channel
  - Annual cost of treatment: 300000$
- 2012: Oxford University, Imperial College London and the University of Edinburgh have launched phase 2 clinical trial for cystic fibrosis patients
- 140 cystic fibrosis patients were randomized to receive placebo (n=62) or 5ml of nebulized gene–liposome complex (n=78; gene therapy) once per month
- The results of the trial:
  - Patients who received gene therapy had significantly higher forced expiratory volume in 1 s (FEV_1) at 12-month follow-up compared to the control group
  - The effect was associated with a stabilization of lung function in gene therapy group
  - No significant difference in treatment-attributable adverse events between groups was observed
  - Further improvements in efficacy and consistency of response to the current formulation are needed before gene therapy is suitable for clinical care

- These findings should encourage the rapid introduction of more potent gene transfer vectors into early phase trials

Duchenne muscular dystrophy

- The **most prevalent** type of **muscular dystrophy** disease in humans
  - 13-33 per 100 000 births (boys only)
- causes **rapid degeneration of muscle tissues** early in life
- the cause: mutations in dystrophin gene
  - The **largest known human gene** (more than **2.6 million bases**), contains **97 exon sequences**
  - **nonsense** or **frameshift** mutations, leading to complete lack of dystrophin, result in Duchenne muscular dystrophy
  - missense mutations are responsible for **Becker** muscular dystrophy (milder form)
- Ataluren
  - A drug for ~15% of patients, carrying nonsense mutation
  - The drug binds to ribosome enabling it to continue translation despite the premature STOP codon
  - Clinical trial confirmed the positive effect in boys with DMD (significant increase in 6-minute walk distance)
- Gene therapy
  - very long coding sequence (~11 kb) – too much for AAV, which could be a perfect vector (serotypes with muscle tropism)
  - micro- / mini- dystrophin: much shorter, functional gene
  - preclinical study on dogs resulted in very promising outcomes

Silencing of genes

RNA interference

- a natural process of degradation or expression inhibition of target transcript by specific short RNA molecules (**siRNA** or **miRNA**).
  - It protects the genome from viruses and regulates the expression of genes

Hereditary amyloidosis

- Caused by mutations in transthyretin gene (TTR)
  - synthesized in the liver, transports thyroxine and vitamin A
  - the mutated protein is accumulated in the form of amyloid (heart, peripheral nerves)
- siRNA complement to 3'UTR of TTR gene (Onpattro)
  - 225 patients with stage 1 or 2 amyloidosis
  - The primary efficacy endpoint was the change from baseline to 18 months in modified **Neuropathy Impairment Score +7** (mNIS+7)
- **Neuropathy Impairment Score +7** (mNIS+7)
  - a composite measure of motor, sensory, and autonomic polyneuropathy including assessments of motor strength and reflexes, quantitative sensory testing, nerve conduction studies, and postural blood pressure

        - Score ranging from 0 to 304; increasing score indicates worsening impairment
            - placebo: score increased by 28 points (worsening)
            - siRNA: score decreased by 6 points
    - Precaution
        - Serum TTR reduction by ~80%
        - May cause vitamin A deficiency (TTR is a carrier for retinol binding protein)
            - Retinol binding protein reduced by 45%; serum vitamin A reduced by 62% during clinical trial
        - Patients should take oral supplementationf of vitamin A (~2500 IU daily)
    - Onpattro
        - registered in EU on 27.08.2018
        - Single dose: 9500$
        - Cost per year: 345k to 450k $

CRISPR

Definition

- Clustered Regularly Interspaced Short Palindromic Repeats
- Prokaryote equivalent for RNAi
    - Provides protection from viral DNA
    - bacterial Cas9 nuclease cuts target DNA in a place indicated by specific RNA molecule (gRNA)

Potential application

- Changing gRNA sequence we can program a target seqence in our genome to be cut
- Almost every fragment of our genome can be targeted by Cas9
- Easy *in vitro* application
- Very high specifity (The cut is applied only in the place targeted by gRNA) High transformation efficiency (~70%)
- Many targets can be cut in a single experiment
- CRISPR allows for „genome editing" – there is no need of sophisticated vector systems that would provide sustained expression of gRNA and Cas9
    - Only temporary expression is required

CRISPR-mediated gene knockout

- The cut is repaired in a proces called Non-Homologous End Joining (NHEJ)
- Error-prone ➔ introducing mutation to target site

CRISPR for expression activation/inactivation

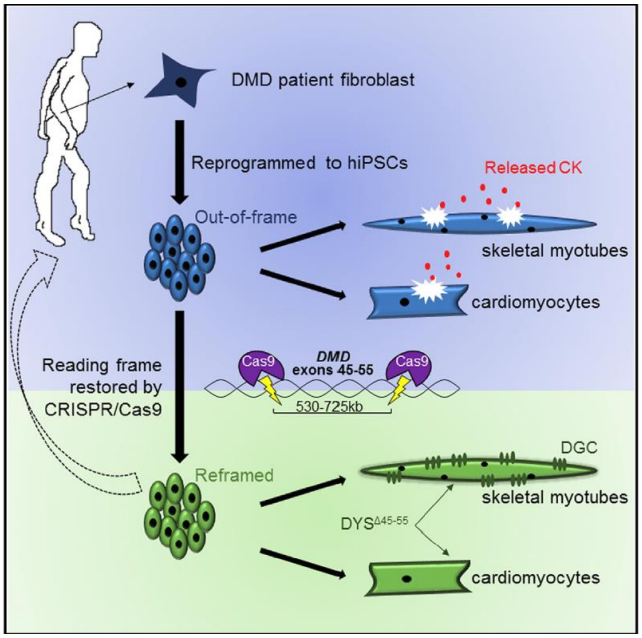
- dCas9: „dead Cas9"
- DNA binding, no nuclease activity
- Fusing dCas9 with transcription repressors/activators, targeting promoter sites

Repair of target sequence with CRISPR

- HDR (Homology Directed Repair), using a DNA template with correct, nonmutated gene sequence

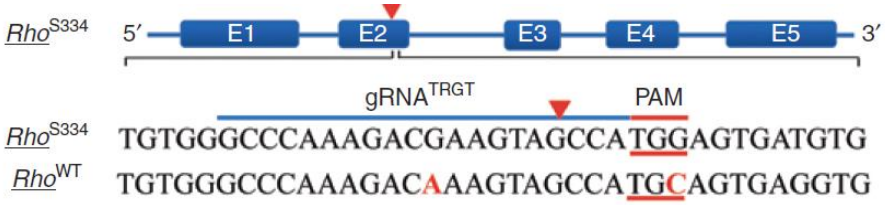
CRISPR – DMD

- 60% of mutations responsible for DMD – exons 45-55
- Using CRISPR to cut out these exons
- As a result – almost fully functional shorter dystrophine
- *in vitro* study confirmed that DMD pluripotent stem cells with 45-55 exons removed by CRISPR-CAS9 can differentiate into healthy skeletal myotubes

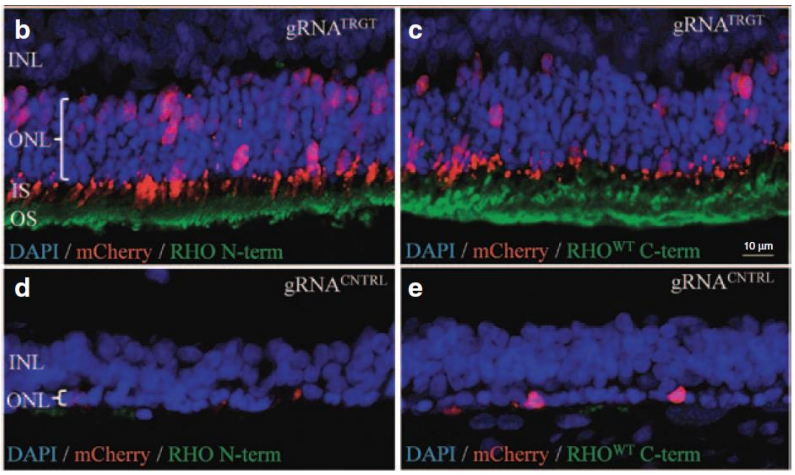


CRISPR – retinitis pigmentosa

- Loss of vision due to progressive loss of rod photoreceptor cells in the back of the eye
- Autosomal dominant disorder
    - Gain of function mutation in rhodopsin gene (*Rho*^{S334})
- Preparation of gRNA targeting mutated dominant allele
    - Introduction of mutations after DNA is cleaved and repaired ➔ conversion to recessive allele



    - Most patients – heterozygotes, having a wildtype allele
- A study on rats revealed that photoreceptors of treated animals significantly improved (up to 8 layers of regenerated photoreceptors vs only one thin layer in the control group)



CRISPR – Huntington's disease

- caused by a **triplet repeat mutation** in the coding region of the **huntingtin** gene (expansion of CAG triplet, encoding glutamin)
    - Autosomal dominant disorder
- **Mutant** huntingtin proteins contain regions where the amino acid **glutamine can be repeated hundreds of times** (the treshold is **>35 repeats**)
    - Such mutated protein cannot be metabolised ➔ accumulation of aggregates in the brain
- CRISPR/Cas9 – cutting-out the fragment encoding poly-glutamine chain
- animal model (mouse)
    - 140 Gln repetitions in HTT gene
    - 9-10 month old mouse have huntigtin aggregates in the brain
    - Two AAV vectors (injected to striatum)
        - 2 gRNAs (T1, T3) under U6 promoter
        - Cas9 under CMV promoter
    - Significant reduction of huntigtin content in the cells
    - Significant imporvement in motor functions