CRISPR GENE EDITING: FROM TAILORED GENE THERAPY TO SPECIES ENGINEERING

Tapani Ronni, PhD

About the Speaker

- **PhD in Genetics, University of Helsinki, Finland**
- **Postdoctoral fellow, University of California, Los Angeles**
- **Scientific interests: gene therapy, microbiology, immunology**
- **A full time medical translator since 2007 (English-Finnish)**

Contents of This Talk

- Human genome and genetic diseases
- The concept of gene therapy
- A brief overview of the CRISPR-Cas system
- CRISPR-Cas as a tool for basic research
- CRISPR-Cas as a tool for gene therapy
- Cancer gene therapy
- Gene editing in human embryos
- Species engineering

The human genome

- **3 billion (3×10^9) base pairs**
- **46 chromosomes (22x2 plus XX or XY)**
- **Fully sequenced**
- **Large personal variations between people**
- **Particular mutations can be beneficial or harmful**
- **Genetic disorders**

Genetic disorders

- **At least 3000 to 4000 genes are known to be associated with phenotypic traits or genetic disorders**
- **Mutations range from one nucleotide change to large deletions or insertions**
- **Palliative treatment usually only option**
- **Occasionally, protein therapy as well**

Protein therapy

- Patients get purified form of protein they are missing or that they cannot produce correctly
- Example: Gaucher disease
  - Lipid storage disorder
  - Recombinant glucocerebrosidase enzyme given by infusion for life
  - Price: $200,000/year
- Good results but extremely expensive
  - Gene therapy candidate

Recessive vs. dominant

- **Gaucher disease is autosomal recessive – disease manifests if both copies of the gene are defective**
- **Some diseases are autosomal dominant – one defective copy is enough**
  - **Huntington's disease**

What is gene therapy?

- **An experimental technique that uses genes to treat or prevent disease**
- **Correct the genetic defect in patients suffering from a genetic disorder**
- **Multigenic diseases too complicated**
- **Monogenic diseases more amenable targets**

What is gene therapy (cont.)?

- **Genetic information resides in the nucleus**
  - **Need to move the desired genetic material there**
- **Transduction of mammalian cells**
- **At least 1% of cells need transduction for gene therapy to work – depends on the condition and cell type**

What is gene therapy (cont.)?

- **How to transduce cells?**
  - **Liposomes**
  - **Nanoparticles**
  - **Nucleofection**
  - **Tailored viruses**

History of Gene Therapy

- First proposed in 1972
- First transgenic animals in 1982
- First attempt in 1990 (SCID)
- Gelsinger case in 1999 was a big setback
- Field has since rebounded with better safety regulations

Gene Therapy Vectors

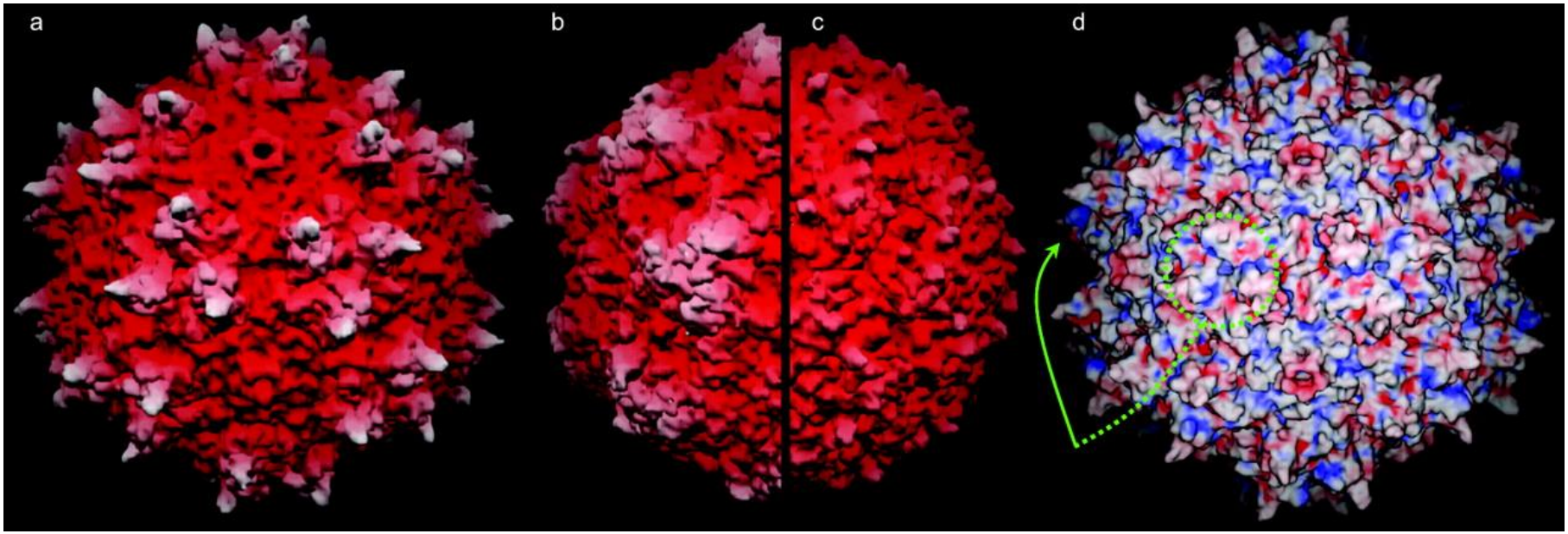
- **Only one virus type (AAV) discussed here as it is most promising as a vector**
- **Viruses have a natural ability to enter target cells**
- **Viruses can be modified by genetic engineering -> novel target specificities and safety features**

Gene Therapy Vectors: Adeno Associated Virus

- **Small, ssDNA virus**
- **Mostly non-immunogenic**
- **Infect broad variety of cells, incl. non-dividing cells**
- **Recombinant AAV vectors don't integrate their DNA into genome**
- **However, insert size max. 4 kb**

Gene Therapy Vectors: Adeno Associated Virus (cont.)

25 nm



AAV-2 Canine parvovirus Insect densovirus

Xie Q et al. PNAS 2002;99:10405-10410

Targets of Gene Therapy

- **Somatic cells vs. germ cells**
- ***Germ cells are eggs and sperm***
  - ***Haploid gene content***
- **Somatic cells are all the cells that are not germ cells**
- **All current trials are targeting somatic cells**

Targets of Gene Therapy (cont.)

- ***Ex vivo* vs. *in vivo* gene therapy**
- ***Ex vivo* is historically older and easier**
  - **Remove cells from the patient, engineer them, select & grow and give back**

- **In vivo means delivering the vector into the patient tissue in the body**
  - **Solid organs (lung, brain)**
  - **Vector immunogenicity?**
  - **Monitoring of efficacy and off-target effects?**

Gene augmentation vs. gene editing

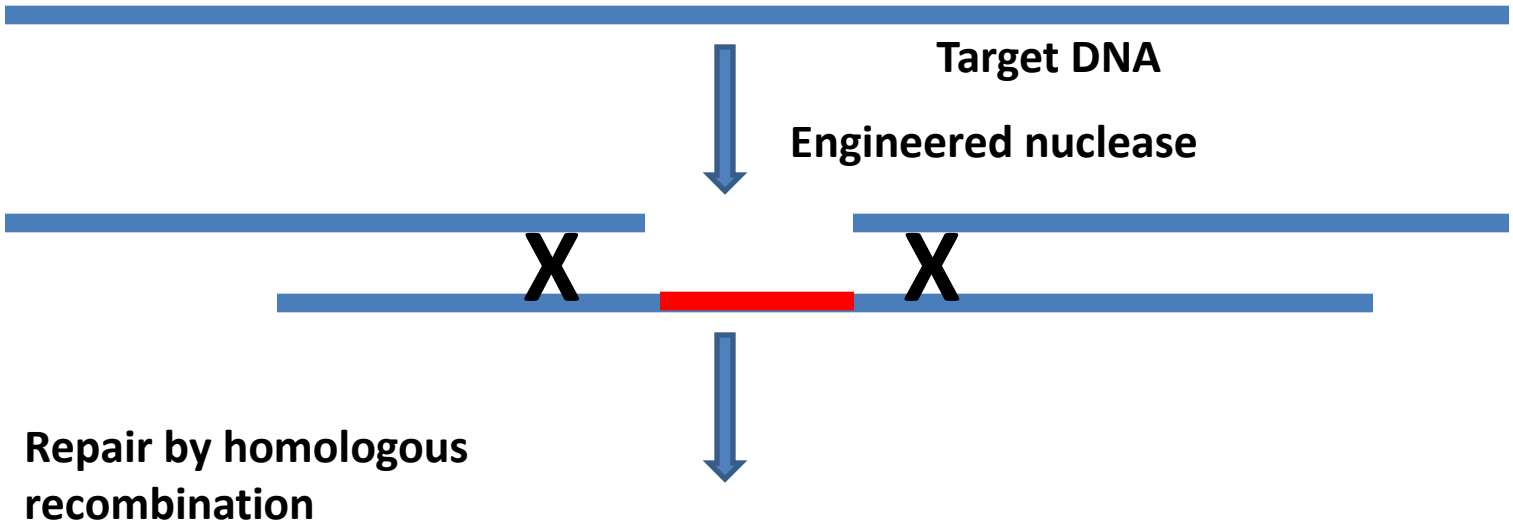
- ***Gene augmentation*: healthy gene transferred into cell with a defective gene**
  - **Hope to overcome the defect in phenotype**
  - **Works best with recessive mutations**
  - **Not effective with multigenic diseases or chromosomal abnormalities**

- ***Gene editing* was science fiction until recently**
  - **Host genome targeted using nuclease-recombination systems**
  - **Optimally, the defective DNA sequence is replaced with a correct one**

CRISPR-Cas: Acquired Immunity in Bacteria

- **Microbes have to survive in hostile environments full of viruses and foreign DNA**
- **They have to differentiate between ”foreign” and ”self” DNA**
- **Restriction enzymes cleave foreign (viral) DNA; RE have been used for decades in genetic engineering**
- **Recently, the CRISPR-Cas system has been discovered and studied**
  - **[CRISPR = Clustered regularly interspaced short palindromic repeats]**

Genome Editing using Homologous Recombination



Corrected point mutation
(e.g. with Sickle Cell Anemia)

Functional gene correction
(e.g. with SCID-X1)

Safe harbor gene addition
(e.g. with SCID-X1)

CRISPR-CAS as a Tool for Basic Research

- Genome editing used to be slow and laborious
- Now a grad student can do it in weeks
- New tailored cell lines to investigate basic cell biology or cancer development
- New engineered mouse strains
- Interactions of multiple genes
- Genome-wide screenings (system biology)

CRISPR-CAS as a Tool for Gene Therapy

- **More accurate, more convenient, less time**
- ***Ex vivo* vs. *in vivo* targets**
- ***Ex vivo*: remove cells, edit, give back to the patient**
- ***In vivo*: edit cells in situ (e.g. brain, muscle)**

CRISPR-CAS as a Tool for Gene Therapy (cont'd)

- **Proof of concept: Duchenne muscular dystrophy**
  - **A fatal muscle disease, caused by a genetic defect in dystrophin gene**
    - **Dystrophin protein dysfunctional**
  - **3 papers in Science (January 2016) showed that dystrophin gene can be edited in vivo in a mouse model of Duchenne, using AAV and CRISPR-Cas**
  - **Restoration of a functional protein**

Safety Concerns With Gene Editing

- **Gene editing is exciting BUT:**
  - **Off-target mutagenesis?**
  - **Donor DNA targeted randomly into genome could lead to cancer**
  - **Make sure that targeted sequence is unique in the genome**
- **FDA is willing to accept some risk**

Efficacy Concerns With Gene Editing

- Enough of the target cells need to be altered for clinical benefit
- Depends on the disease and target
  - Hematopoietic stem cells
  - Fitness advantage helps
  - Some diseases need only a small increase of healthy protein for good outcome
    - Blood clotting diseases

Regulatory framework for gene therapies

- Food and Drug Administration (FDA)
  - Center for Biologics Evaluation and Research (CBER)
    - Office of Cellular, Tissue and Gene Therapies
- In Europe, European Medicines Agency

Cancer Gene Therapy

- Attack cancer cells directly by gene editing
- Alter immune cells of the body so they kill cancer cells better
- A hot new field: CAR-T
  - Gene editing of T cells so they express new chimeric antigen receptors specific for cancer target
  - Tailored therapy for each patient
  - Safety vs. efficacy

Gene Editing in Human Embryos

- Done in China with non-viable embryos
- Ethical issues are huge
  - Informed consent?
  - Germ line altered
  - Off-target effects in future generations
- Risks vs. benefits need to be understood better
- Legal framework does not allow genetic alteration of humans

Species Engineering

- **Concept: alter the genome of an individual of the species of interest and spread the alteration into the target population**
- **Gene Drive with CRISPR-Cas**
- **Very slow with mammals, but shown to work fairly quickly with insects**

Species Engineering (cont'd)

- **Gene Drive alters the Mendelian inheritance pattern of a given allele**
- **CRISPR-Cas construct copies itself to the other member of the chromosome pair in diploid organisms**
- **Transmission rates 99.6% in one experiment with the malaria mosquito**

Future Prospects

- **Cancer gene therapy**
  - **Make cancer cells self-destruct or turn more benign**
  - **Boost the immune system by altered T cells**
- **Gene Editing therapies have exponential growth potential once the technical difficulties and pricing issues have been sorted out**
- **Germ line therapy problematic**

Future Prospects

- **Species engineering**
  - **Destroy malaria mosquito populations in Africa?**
  - **Side effects in ecosystems?**
- **Once the species engineering experiment has started in the wild one can only wait and see**
- **Is it ethical to tailor entire species?**

References

1. 1000 Genomes Project Consortium, Abecasis GR, Auton A, et al. An integrated map of genetic variation from 1,092 human genomes. Nature. 2012 Nov 1;491(7422):56-65. doi: 10.1038/nature11632.

2. Li, HL, Nakano, T, Hotta, A. Genetic correction using engineered nucleases for gene therapy applications. Dev Growth Differ. 2014 Jan;56(1):63-77. doi: 10.1111/dgd.12107. Epub 2013 Dec 11.

3. http://ghr.nlm.nih.gov/handbook/therapy/genetherapy. Accessed October 14, 2014.

4. Hille, F, and Charpentier, E. CRISPR-Cas: biology, mechanisms and relevance. Philos Trans R Soc Lond B Biol Sci. 2016 Nov 5;371(1707). pii: 20150496. doi: 10.1098/rstb.2015.0496.3.

5. La Russa, MF, and Qi, LS. The New State of the Art: Cas9 for Gene Activation and Repression. Mol Cell Biol. 2015 Nov;35(22):3800-9. doi: 10.1128/MCB.00512-15. Epub 2015 Sep 14.

6. Friedmann T, Roblin R. Gene therapy for human genetic disease? Science. 1972 Mar 3;175(4025):949-55.

7. Carter, JB and Saunders VA. *Virology: Principles and applications. 2nd ed.* Wiley; 2013.

8. Tabebordbar M, Zhu K, Cheng JK, et al. In vivo gene editing in dystrophic mouse muscle and muscle stem cells. Science. 2016 Jan 22;351(6271):407-11. doi: 10.1126/science.aad5177. Epub 2015 Dec 31.

9. Nelson CE, Hakim CH, Ousterout DG, et al. In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy. Science. 2016 Jan 22;351(6271):403-7. doi: 10.1126/science.aad5143. Epub 2015 Dec 31.

10. Long C, Amoasii L, Mireault AA, McAnally JR, et al. Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. Science. 2016 Jan 22;351(6271):400-3. doi: 10.1126/science.aad5725. Epub 2015 Dec 31.

11. Chew WL, Tabebordbar M, Cheng JK, et al. A multifunctional AAV-CRISPR-Cas9 and its host response. Nat Methods. 2016 Oct;13(10):868-74. doi: 10.1038/nmeth.3993. Epub 2016 Sep 5.

12. Akbari, OS, Bellen, HJ, Bier, E, et al. Safeguarding gene drive experiments in the laboratory. Science. 2015 Aug 28;349(6251):927-9. doi: 10.1126/science.aac7932. Epub 2015 Jul 30.

13. Hammond A, Galizi R, Kyrou K et al. A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae. Nat Biotechnol. 2016 Jan;34(1):78-83. doi: 10.1038/nbt.3439. Epub 2015 Dec 7.

14. Morris, EC, and Stauss, HJ. Optimizing T-cell receptor gene therapy for hematologic malignancies. Blood. 2016 Jun 30;127(26):3305-11. doi: 10.1182/blood-2015-11-629071. Epub 2016 May 20. Review.

15. Newick, K, Moon, E, and Albelda, SM. Chimeric antigen receptor T-cell therapy for solid tumors. Mol Ther Oncolytics. 2016 Apr 13;3:16006. doi: 10.1038/mto.2016.6. eCollection 2016. Review.

16. Liang, P., Xu, Y., Zhang, X, et al. CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. Protein Cell. 2015 May;6(5):363-72. doi: 10.1007/s13238-015-0153-5. Epub 2015 Apr 18.

17. Smith KR, Chan S, Harris J. Human germline genetic modification: scientific and bioethical perspectives. Arch Med Res. 2012 Oct;43(7):491-513.

Thank you!

tapanironni@yahoo.com