CRISPR/Cas9-Based Gene Therapy for Duchenne Muscular Dystrophy

Charles A. Gersbach, Ph.D.
Department of Biomedical Engineering
Department of Orthopaedic Surgery
Center for Genomic and Computational Biology
Duke University

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Challenges for Gene Therapy

1. Safety
   Inability to control transgene-genome interactions

2. Delivery
   Inability to achieve persistent gene expression

3. Efficiency
   Inability to deliver large genes
Challenges for Gene Therapy

1. Safety
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All potentially addressable by correcting endogenous gene (Genome Editing)

Gene Therapy for DMD

DNA → mRNA → Protein

Adeno-Associated Virus: Extra copy of the dystrophin DNA
   - **Challenge**: Large dystrophin gene

Exon Skipping: Restore expression from the mRNA
   - **Challenge**: Requires continuous administration

Genome Editing: Restore expression from the native gene
   - **Challenge**: Many…
Duchenne Muscular Dystrophy

- Occurs 1/3500 male births
- Debilitating during childhood & death during 20’s
- Respiratory complications & cardiac myopathy
- Inherited or spontaneous mutation to dystrophin
  - 79 exons over 2.5 Mb (14 kb cDNA)
- Cytoskeletal structural protein
  - Cell integrity & intracellular signaling
- No current therapeutic options!

Extracellular Matrix
- Dystrophin Glycoprotein Complex
- Dystrophin
- Actin

Genome Editing with Engineered Nucleases

Target Gene + Nuclease(s)

- Gene Addition/Exchange (Homologous Recombination)
- Gene Disruption (Non-Homologous End Joining)
- Gene Deletion (Non-Homologous End Joining)

Correction of Genetic Diseases by Genome Editing:

Programmable Nucleases

Zinc Finger Nucleases (ZFNs) and TALENs

DNA-binding domains:
• Zinc finger proteins
• TAL effectors

Effector domains:
• FokI endonuclease catalytic domain

CRISPR/Cas9

Restoring Dystrophin Expression around Exon 44-50 Deletion Hotspot

<table>
<thead>
<tr>
<th>DMD genotype</th>
<th>Dystrophin mRNA transcript</th>
<th>Resulting dystrophin protein</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>44 45 46 47 48 49 50 51 stop 52</td>
<td></td>
<td></td>
<td>Normal</td>
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<tr>
<td>44</td>
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• Exon 51 skipping can correct 13% of DMD mutations
• Oligonucleotide-mediated exon skipping is successful in clinical trials (Lancet, N Engl J Med, March 2011)
  • Requires lifelong treatment once a week
• Goal: Restoration by genome editing
Restoring Dystrophin Expression around Exon 44-50 Deletion Hotspot

**DMD genotype**

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After correction:

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Dave Ousterout

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Editing the Dystrophin Gene with CRISPR/Cas9

Target: Exon 51

<table>
<thead>
<tr>
<th>Cas9 gRNA</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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% indels: 5.0 4.0 6.8 9.7 5.3

CR1/5 treated genomic DNA

CR2/5 treated genomic DNA

Genomic DNA

Precise deletion of exon 51 from the genome

Exon 51 skipping can correct 13% of DMD mutations (Phase III trials)

• Skipping 45-55 can correct 62% of DMD mutations (Aartsma-Rus et al., *Hum Mutat* 2009)
• Multi-exon skipping in preclinical development (Aoki et al., *PNAS* 2012)
Genome Editing for Duchenne Muscular Dystrophy

Delivery challenges

Autologous cell-based gene therapy
- Skeletal myoblasts
- Mesoangioblasts
- CD133+ cells
- Bone marrow stem cells
- Pericytes/MSCs
- Dermal fibroblasts
- Induced pluripotent stem cells (Darabi et al., Cell Stem Cell 2012; Filareto et al., Nat Comm 2013)

Delivery of nucleases to skeletal and cardiac muscle 

Cell-Based Therapies for Muscle Disorders

Human Spectrin

Human Dystrophin

DMD untreated

DMD/Δ51 corrected clone

Human skeletal myoblast transplantation into NOD/SCID/γc mice

Ousterout et al. Mol Ther (2014)
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Delivery of nucleases to skeletal and cardiac muscle in vivo

Summary
- Genome editing for Duchenne Muscular Dystrophy
- Multiple strategies for correcting reading frame
- Restoration of dystrophin expression in myoblasts from DMD patients
- No toxicity and limited off-target activity
- Robust gene editing, dystrophin restoration, and improved function following in vivo delivery

Challenges:
- Safety
- Immunogenicity
- Delivery & Efficiency
- Progenitor cells

General tool for science and medicine
Gersbach Lab
Josh Black
Jonathan Brunger
Matt Gemberling, PhD
Tyler Gibson, PhD
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Hunter Hutchinson
Ami Kabadi, PhD
Tyler Klann
Dewran Kocak
Feimei Liu
Josh McMenemy
Christopher Nelson, PhD
David Ousterout, PhD
Pablo Pérez-Piñera, MD, PhD
Adrian Pickar, PhD
Adrianne Pittman
Lauren Polsteinm PhD
Jacqueline Robinson-Hamm
Nishkala Shivakumar
Pratiksha Thakore

Collaborators
Farshid Guilak (Duke)
Greg Crawford (Duke)
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Thank You