Emerging Treatment Strategies for FSHD

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Co-Principal Investigators
Disclosures:
Peter Jones and Takako Jones are listed as inventors on US patent applications for epigenetic diagnosis of FSHD, epigenetic therapeutic targets and CRISPR therapy for FSHD.

Peter Jones is on the SAB for Fulcrum Therapeutics and receives financial compensation.
Rare Diseases as a group are not so rare
>90 Neuromuscular Diseases

~30 muscular dystrophies: progressive weakness and degeneration of the skeletal muscles that control movement.

Muscular dystrophy: 9 classes of disease
- BMD (Becker)
- DMD (Duchenne)
- EDMD (Emery-Dreifuss)
- LGMD (Limb-Girdle)
- OPMD (Oculopharyngeal)
- CMD (Congenital)
- DDM (Distal)
- FSHD (Facioscapulohumeral)
- MMD (Myotonic)

Aging can be considered a muscle disease
Big Picture Perspective

Research across the full spectrum of neuromuscular diseases leads to scientific and medical breakthroughs that accelerate treatments and cures. The power in this approach is that we can often apply learnings from one disease to progress in others to bring urgently-needed answers to affected patients and families.
Jones Lab expertise is epigenetics and developmental biology.

Since 2003, our focus has been on FSHD, which we now know is an epigenetic-based disease.
Epigenetics

“Treasure your exceptions.”
Thomas Hunt Morgan

- Non-Mendelian pattern of heritability
- Context-dependent sequence independent gene expression
- Can be influenced by the environment (diet, aging, etc…)
Epigenetic differences can have profound long-term health consequences

Epialleles

Genetically identical
Epigenetically different


$\alpha^{IAp}$ allele, methylated

Brown, normal

$\alpha^{IAp}$ allele, unmethylated

Yellow, obese, spontaneous tumors

Affects long-term health $\Rightarrow$ heritable?
All types of FSHD are linked to epigenetic status of 4q35 D4Z4

C. Himeda et al 2014 Antiox Redox Sig
T. Jones et al. 2015 Clinical Epigenetics
# FSHD Therapeutic Development in 2003

<table>
<thead>
<tr>
<th>Feature</th>
<th>Status</th>
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<tbody>
<tr>
<td>FSHD gene?</td>
<td>Unknown</td>
</tr>
<tr>
<td>Pathogenic mechanism?</td>
<td>Unknown</td>
</tr>
<tr>
<td>Cellular models?</td>
<td>Not significant</td>
</tr>
<tr>
<td>Animal models?</td>
<td>Non existent</td>
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**Treatments:** Steroids, myostatin inhibition  
**Rationale:** ~work for DMD, so why not
FSHD Therapeutic Development in 2017

FSHD gene? DUX4

Pathogenic mechanism? Epigenetic dysregulation
Still many possibilities

Cellular models? Many

Animal models? Mice, Fly, Zebrafish

Treatments: Myostatin inhibition, immune suppression
Rationale: FDA approved, basis in the biology
FSHD in 2017
Many viable therapeutic approaches!

1 & 4 Small molecule inhibitors; CRISPR technology → prevent expression of DUX4

2 Anti-sense, morpholinos, PMOs, microRNAs → inactivate or destroy the DUX4-fl mRNA

3 Small molecule inhibitors → block downstream pathogenic effects of DUX4-FL protein (immune suppression; aTyr trial)

4 DUX4-independent approaches
   → Myostatin inhibition (Acceleron ACE-083 trial)
FSHD in 2017
Many viable therapeutic approaches!

Jones Lab at UNRSOM

- Small molecule epigenetic effectors
- CRISPR/dCas9 silencing
FSHD is an epigenetic disease

Can we therapeutically dial down DUX4 expression?

<table>
<thead>
<tr>
<th></th>
<th>DUX4-fl Expression</th>
<th>D4Z4 DNA Methylation</th>
<th>Epigenetically Inducible</th>
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<tbody>
<tr>
<td>Healthy</td>
<td>-/-/+</td>
<td>High</td>
<td>-/+</td>
</tr>
<tr>
<td>FSHD1-affected</td>
<td>+++</td>
<td>Low*</td>
<td>+++</td>
</tr>
<tr>
<td>FSHD1-nonmanifesting</td>
<td>-/++++</td>
<td>Low-Mid*</td>
<td>++</td>
</tr>
<tr>
<td>FSHD2</td>
<td>+++</td>
<td>Very Low**</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N = 11-~100 RU, Ave ~28 RU
N = 1-10 RU
Reported in N = 5-10 RU
N = >10 RU, Ave ~16 RU

= More repressed chromatin
= More relaxed chromatin
= Intermediate chromatin states
= Hypomethylated CpGs
= Hypermethylated CpGs
= Intermediate methylation status

T Jones et al. 2015 Clinical Epigenetics
Epigenetic regulation at chrom 4q35 is distinct between healthy and FSHD

Candidate targets for inhibitory small molecules

FSHD is an epigenetic disease with numerous potential therapeutic targets

Asymptomatic \(\leftrightarrow\) \(\leftrightarrow\) \(\leftrightarrow\) \(\leftrightarrow\) \(\leftrightarrow\) \(\leftrightarrow\) \(\leftrightarrow\) \(\leftrightarrow\) FSHD
We have identified 3 strong candidates for targeted FSHD therapy

Example: Epigenetic Regulator PT-2

Design small molecule inhibitors to reverse the epigenetic state
Targeted repression of key epigenetic regulators reduces pathogenic *DUX4* expression

Epigenetic drugs are a viable therapeutic approach to FSHD
FSHD is gain-of-function

Can we therapeutically dial down DUX4 expression using CRISPR?

T Jones et al. 2015 Clinical Epigenetics
CRISPR-mediated “genome editing” 
Powerful, controversial, scary?

Not the whole story
CRISPR is much more than genome “editing”

CRISPR/Cas technology is essentially a simple and more efficient way to specifically target the genome of any organism.

Sequence-specific genome targeting
CRISPR/dCas9 in FSHD therapeutic development

Efficient genome targeting of a transcriptional repressor

Himeda et al. (2015) Mol. Therapy
 Proof-of-principle CRISPR “cure” for FSHD
Could CRISPR really become an FSHD therapeutic?

Three companies have had recent IPOs
Other companies still privately held
Projected market of $5.5 billion by 2021
Patent* is being contested: UC-Berkeley vs the Broad Institute*
New CRISPR and CRISPR-like systems being discovered
Therapeutic delivery of CRISPR/Cas in vivo is challenging

FSHD is a skeletal muscle disease

Need an animal model → pathogenic gene is primate-specific
Inserted the human *DUX4* gene into the mouse genome to generate FSHD-like mice with a readily assayable phenotype.

- *FLEx/+* (control)
- *FLEx/+*, *ACTA1 MCM* (no *DUX4*)
- *FLEx/+*, *ACTA1 MCM* (DUX4 induced)

Mouse 1: >1 min suspended
Mouse 2: >1 min suspended
Mouse 3: <2 second suspended

*FLEx/+*, *ACTA1 MCM* (DUX4 induced)
In vivo delivery of AAV9-dCas9-KRAB + AAV9-sgRNA leads to significant DUX4 knockdown

AAV9 delivery results in 30% decrease in TA muscle

Enough to be therapeutic?

Only need to dial back expression from affected to asymptomatic
Our recent increased understanding of FSHD pathogenic mechanisms has led to the development of numerous therapeutic approaches and tools.

CRISPRi/dCas9-KRAB; CRISPR/Cas9; Myostatin inhibition; Morpholinos/PMOs/shRNAs; miRNAs; Anti-inflammatory; Small molecules targeting epigenetic regulators; more…
The FSHD field will be translating discoveries to the clinic and the future is bright

Steven Blier: Concert pianist, Professor at Julliard, FSHD patient, and friend
Kelli O’Hara: Tony award winning actress and advocate for FSHD
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