Emerging Treatment Strategies for FSHD

LA patient meeting Oct 21, 2017

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

Peter Jones is on the SAB for Fulcrum Therapeutics
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) | CMD (Congenital) |
| DMD (Duchenne) | DDM (Distal) |
| EDMD (Emery-Dreifuss) | FSHD (Facioscapulohumeral) |
| LGMD (Limb-Girdle) | MMD (Myotonic) |
| OPMD (Oculopharyngeal) |

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.
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2002 Patient meeting: FSHD is caused by a loss of epigenetic regulation

Ryan Wuebbles, PhD

Introduced us to facioscapulohumeral muscular dystrophy
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...)
The gene “environment” affects gene expression

Translocation of a gene from a euchromatic region to a heterochromatic region resulting in inactivation of nearby euchromatic genes

Normal Genetic mutant Epigenetic mutant

Translocation of a gene from a euchromatic region to a heterochromatic region resulting in inactivation of nearby euchromatic genes

heterochromatin

ON

Inversion ($w^{m1}$)

OFF
Epigenetic differences can have profound long-term health consequences.

### Epialleles

- **Genetically identical**
- **Epigenetically different**


Affects long-term health

→ heritable?
FSHD is an epigenetic disease

The FSHD gene, *DUX4*, is under epigenetic regulation

The “genetic environment” is changed in FSHD
Jones Lab expertise is epigenetics and developmental biology of FSHD

Since 2003, our focus has been on FSHD pathogenic mechanisms, therapeutic targets, and animal models.

FSHD is caused by a loss of epigenetic regulation

Ryan Wuebbles, PhD
# FSHD Therapeutic Development in 2003

<table>
<thead>
<tr>
<th>Question</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, biology-based
FSHD in 2017
Many viable therapeutic approaches!

Anti-sense, morpholinos, PMOs, microRNAs
\(\rightarrow\) inactivate or destroy the DUX4-fl mRNA
FSHD in 2017
Many viable therapeutic approaches!

Small molecule inhibitors; CRISPR technology
→ prevent expression of DUX4
FSHD in 2017
Many viable therapeutic approaches!

Small molecule inhibitors
→ block downstream pathogenic effects of DUX4-FL protein
FSHD in 2017
Many viable therapeutic approaches!

**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 return to an FSHD non-affected epigenetic state?

T Jones et al. 2015 Clinical Epigenetics
The FSHD genetic region normally is bound by negative regulators, in FSHD it is bound by positive regulators.
The FSHD genetic region normally is bound by negative regulators, in FSHD it is bound by positive regulators.

Healthy and Asymptomatic

Epigenetic drugs are a viable therapeutic approach to FSHD
We have identified strong candidates for targeted FSHD therapy

Knockdown this regulator returns the region to healthy state

Example: Epigenetic Regulator PT-2
Targeting FSHD epigenetics

Can we therapeutically return to a non-affected epigenetic state by recruiting OFF machinery?

T Jones et al. 2015 Clinical Epigenetics
The FSHD gene normally is bound by negative regulators, in FSHD it is bound by positive regulators.

Healthy and Asymptomatic

OFF OFF

X

FSHD

CRISPR technology as a therapeutic approach to FSHD
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 any sequence of the genome of any organism.

Sequence-specific genome targeting
- Cut the DNA → editing
- Target an activator → turn a gene “ON”
- Target a repressor → turn a gene “OFF”
- Target a tag → “see” a gene, capture a gene
Can we use CRISPR technology as a therapy for FSHD?

Charis Himeda, PhD

CRISPR/Cas9 Editing

CRISPR/dCas9 Transcription Modulation

FSHD

Ch. 4q35

D4Z4

D4Z4

D4Z4

DUX4

AAAAAA

(D3 (PAS))

Promoters, Enhancers, Gene bodies

sgRNA

dCas9

dCas9-VP16

Upregulated Utrophin mRNA


Himeda et al. (2015) Mol. Therapy
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
CRISPR/dCas9-mediated Transcriptional Inhibition Ameliorates the Epigenetic Dysregulation at D4Z4 and Represses DUX4-fl in FSH Muscular Dystrophy

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Proof-of-principle CRISPR “cure” for FSHD

How CRISPR could lead to a cure for muscular dystrophy

How Controversial Gene Editing Could Lead To Groundbreaking Cures
Could CRISPR really become an FSHD therapeutic?

Projected market of $5.5 billion by 2021

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 $\Rightarrow$ pathogenic gene is primate-specific.

Maeder and Gersbach (2016) Mol Ther 24:430
Generation of a viable, phenotypic FSHD-like mouse based on DUX4-fl expression

Takako Jones, PhD

FLExDUX4

The Rosa26 promoter ensures robust DUX4-fl expression in all cells that underwent cre-mediated inversion
FLEXDUX4 model mice show rapid decline in mobility and a severe FSHD-like myopathy
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: Musician, Professor at Julliard, FSHD patient, and friend
Kelli O’Hara: Tony award winning actress and advocate for FSHD
Department of Pharmacology

Acknowledgements

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