Numerous therapeutic avenues for FSHD

Himeda et al. (2015) Antiox Redox Signaling
Epigenetics

“Treasure your exceptions.”

Thomas Hunt Morgan

- Context-dependent sequence independent gene expression
- Stable yet dynamic \(\rightarrow\) Cellular memory
- Can be influenced by the environment (diet, aging, etc…)
FSHD genetics are complex

Himeda et al. (2014) Antiox Redox Signaling
FSHD is primarily an epigenetic disease

High variability within the clinical and genetic population

→ Presentation, progression, severity, asymmetry, extramuscular, and age of onset

De Greef et al. (2009) Human Mutation
All types of FSHD are linked to epigenetic status of 4q35 D4Z4

FSHD1: Dominant deletions at 4q35 D4Z4 array
   Apparently low penetrance
   DNA Hypomethylation of shortened 4q35

FSHD2: Dominant inactivating mutations in SMCHD1
   --ATPase chromatin remodeling protein
   -- account for ~85% of FSHD2 → more mutations
   DNA Hypomethylation of 4q35 and 10q26 arrays

IFSHD: Infantile form of FSHD1 or FSHD2, much more severe
   DNA Hypomethylation of FSHD1 or 2
Epigenetic Gene and Genome Regulation

“The structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states” – Adrian Bird

Keys: DNA sequence independent
Context dependent
Stable/Heritable
Cellular Memory
Dynamic/reversible
Responsive

Chromatin: DNA, histones, non-histone proteins, RNA
Epigenetics: Nurture (Environment) vs. Nature

Heritable changes in gene activity that do not involve alterations to the genetic code

“Epialleles”

<table>
<thead>
<tr>
<th>Nature: Genetics</th>
<th>vs.</th>
<th>Nurture: Epigenetics</th>
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<tbody>
<tr>
<td>Passed on by Mom and Dad</td>
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<td>Affected by:</td>
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<tr>
<td>• Mutagens</td>
<td></td>
<td>• Stress</td>
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<tr>
<td>• Mistakes</td>
<td></td>
<td>• Prenatal care</td>
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<tr>
<td>Genetically identical</td>
<td></td>
<td>Genetically identical, epigenetically different</td>
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</table>

Epigenetic differences in genetically ~ equivalent people (i.e. identical twins) can have profound effects

Waterland and Jirtle (2003) MCB 23:5293
Epigenetic differences can have profound long-term health consequences.

**Epialleles**

- $A^{IAP}$ allele, methylated
  - Brown, normal

- $A^{IAP}$ allele, unmethylated
  - Yellow, obese, spontaneous tumors

Genetically identical
Epigenetically different

Affects long-term health
⇒ heritable?

FSHD epigenetics dictate disease

Healthy

FSHD1 asymptomatic

FSHD1

FSHD2

\(=\) Hypermethylated CpGs

\(=\) more heterochromatic

\(=\) Hypomethylated CpGs

\(=\) more euchromatic

N= 1-10 RU

N= 5-10 RU

N= 11-26 RU
# Epigenetic Therapies

<table>
<thead>
<tr>
<th>Disease</th>
<th>Epigenetic target</th>
<th>Approaches</th>
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<tr>
<td>FSHD</td>
<td>Euchromatic D4Z4 Chromatin structure</td>
<td>Small molecule inhibitors</td>
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<tr>
<td></td>
<td></td>
<td>CRISPR/Cas9/dCas9</td>
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<tr>
<td><strong>Heterochromatic structure</strong></td>
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<td>Rett Syndrome</td>
<td>Reactivation of silenced X chromosome</td>
<td>Small molecule inhibitors</td>
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<tr>
<td>Fragile X Syndrome</td>
<td>Reactivation of FMR1 gene</td>
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<tr>
<td>Prader-Willi Syndrome</td>
<td>Reactivation of tumor suppressors</td>
<td>Small molecule inhibitors</td>
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<td>Leukemia</td>
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<td>Cancer</td>
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<td>Duchenne MD</td>
<td>Induction of compensatory genes</td>
<td>Small molecules</td>
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<tr>
<td>Myotonic Dystrophy</td>
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<tr>
<td>Emery-Driefuss MD</td>
<td>Nuclear architecture</td>
<td>???</td>
</tr>
</tbody>
</table>
Non-permissive

- Unstable mRNA
- DUX4 protein not made
- Nontoxic

Permissive

- Stable poly A mRNA
- DUX4 protein produced
- Cytotoxic

PAS = polyadenylation site
NP = non-permissive
P = permissive
**DUX4-fl expression is dependent upon the PAS and FSHD/healthy status**

**4qA permissive**

**FSHD**

4qA permissive

DUX4-FL

+++++

**Healthy**

DUX4-FL

+/----

**Nonpermissive**

Healthy

No DUX4

PAS = polyadenylation site  * = translation stop

**Exon 1**

**E2**

**E3**

PAS

AAAAAAA

DUX4-FL

AAAAAAA

DUX4-FL

+/

degraded
degraded

No PAS

No DUX4
Therapeutic approaches to FSHD

Himeda et al. (2015) Antiox Redox Signaling
Epigenetic regulation is a therapeutic target for FSHD

Small molecule inhibitors

FSHD is an epigenetic disease with numerous potential therapeutic targets

Himeda et al. (2015)
Therapeutic approaches to FSHD

Morpholinos/PMOs/shRNAs/miRNAs

CRISPR/Cas9
Anti-inflammatory

CRISPRi/dCas9-KRAB
Myostatin inhibition
CRISPR-Cas9 and dCas9 in muscular dystrophy research and therapeutic development

Efficient genome targeting

CRISPR/Cas9 Editing

**CRISPR/dCas9 Transcription Modulation**

Promoters, Enhancers, Gene bodies

Himeda et al. (2015) *Mol. Therapy*

**FSHD**

Ch. 4q35

D4Z4

sgRNA E-1

sgRNA E-3

sgRNA PAS

DMD: dCas9-VP16 upregulated *Utrophin* mRNA [12]

FSHD: dCas9-KRAB repressed *DUX4* mRNA [7]

Therapeutic delivery of CRISPR/Cas is challenging

Maeder and Gersbach (2016) Mol Ther 24:430