Genetically Similar, Epigenetically Different

Peter L. Jones, Ph.D. and Takako I. Jones, Ph.D.
Principal Investigators

The Wellstone Program
Departments of Cell and Developmental Biology & Neurology
Epigenetics is Increasingly a Focal Area in Human Health and Disease Research

Cocaine can change the brain in ways that can be passed on to male offspring making them less likely to find the drug rewarding or work hard to get it.

**The Epigenome: Guiding Cells to Their Specialized Roles**

Researchers are finding that a complex layer of proteins and markers called the epigenome controls access to genetic information, allowing each cell to read the genes necessary for cell-specific functions but blocking off most of the rest of the genome.
Complexity of an organism

3 x 10^{13} Cells/human

2 x 10^{12} Proteins/cell

\sim 22,000\text{ Protein-coding genes}
  > 90\% \text{ have alternative splicing}

> 40,000\text{ noncoding RNAs}
  > 400,000\text{ regulatory regions}

1\text{ Genome}

Each cell requires a specific complement of gene products

Expression of necessary genes
Repression of unwanted genes
Complexity of an organism

3 x 10^{13} Cells/human

2 x 10^{12} Proteins/cell

\sim 22,000\ Protein\text{-}coding\ genes
  \quad >90\%\ have\ alternative\ splicing

>40,000\ noncoding\ RNAs
  \quad >400,000\ regulatory\ regions

1\ Genome + Epigenome =

\rightarrow\ \text{Organize the nucleus}
\rightarrow\ \text{Affect mRNA content}
\rightarrow\ \text{Integrate the environment}
\rightarrow\ \text{Cellular memory}

Expression of necessary genes
Repression of unwanted genes
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
      Dynamic/reversible
      Responsive

Chromatin: DNA, histones, non-histone proteins, RNA
These mice are genetically identical yet epigenetically different

“Epialleles”

DNA methylation is the classic epigenetic mark
Human DNA methylation is exclusively on CpGs

Art Riggs (1975) CpG methylation as a mechanism for memory

\[
\begin{align*}
5' - \text{C}^\text{mpG} & \quad \text{G} \, \text{p} \, \text{C}-5' \\
\text{M} \quad \text{M} \\
5' - \text{C} \, \text{pG} & \quad \text{G} \, \text{p} \, \text{mC}-5'
\end{align*}
\]
Epigenetic differences can have profound long-term health consequences

Genetically identical
Epigenetically different

Epigenetics: Nurture (vs. Nature)

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

<table>
<thead>
<tr>
<th>Nature: Genetics</th>
<th>vs.</th>
<th>Nurture: Epigenetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passed on by Mom and Dad</td>
<td></td>
<td>Passed on by Mom and Dad</td>
</tr>
<tr>
<td>Affected by:</td>
<td></td>
<td>Affected by:</td>
</tr>
<tr>
<td>• Mutagens</td>
<td>• Prenatal care</td>
<td></td>
</tr>
<tr>
<td>• Mistakes</td>
<td>• Sleep</td>
<td></td>
</tr>
<tr>
<td>• Diet</td>
<td>• Environment</td>
<td></td>
</tr>
</tbody>
</table>

Genetically identical

Genetically identical, epigenetically different

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

Waterland and Jirtle
## Epigenetic Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Epigenetics</th>
<th>Manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSHD</td>
<td>Chromatin structure</td>
<td>Progressive skeletal muscle loss</td>
</tr>
<tr>
<td>Rett Syndrome</td>
<td>MeCP2</td>
<td>Intellectual disabilities</td>
</tr>
<tr>
<td>ATR-X</td>
<td>Snf2 remodeling</td>
<td>Intellectual disabilities, α-thalassaemia</td>
</tr>
<tr>
<td>Fragile X Syndrome</td>
<td>DNA methylation</td>
<td>Intellectual disabilities</td>
</tr>
<tr>
<td>ICF Syndrome</td>
<td>DNA methylation</td>
<td>Immunodeficiency</td>
</tr>
<tr>
<td>Angelman’s Syndrome</td>
<td>LOI</td>
<td>Intellectual disabilities</td>
</tr>
<tr>
<td>Prader-Willi Syndrome</td>
<td>LOI</td>
<td>Obesity, intellectual disabilities</td>
</tr>
<tr>
<td>Beckwith-Wiedemann</td>
<td>LOI</td>
<td>Organ overgrowth</td>
</tr>
<tr>
<td>Leukemia</td>
<td>DNA methylation</td>
<td>Disrupted haematopoiesis</td>
</tr>
<tr>
<td>Lupus</td>
<td>DNA methylation</td>
<td>Chronic inflammation in joints, skin</td>
</tr>
<tr>
<td>Cancer</td>
<td>DNA methylation</td>
<td>Uncontrolled cell cycle</td>
</tr>
<tr>
<td>Rubinstein-Taybi</td>
<td>CBP (HAT)</td>
<td>Intellectual disabilities</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>HDAC?</td>
<td>Autoimmune CNS degeneration</td>
</tr>
<tr>
<td>Spinal muscular atrophy</td>
<td>HDAC?</td>
<td>Motor neuron disease</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>DNA methylation?</td>
<td>Destruction of articular cartilage ECM</td>
</tr>
<tr>
<td>Obesity</td>
<td>DNA methylation</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>DNA methylation</td>
<td></td>
</tr>
<tr>
<td>Bipolar Disorder</td>
<td>DNA methylation</td>
<td></td>
</tr>
</tbody>
</table>
FSHD is linked to D4Z4, the A type subtelomere and the epigenetic status of the 4q35 D4Z4 repeat

Healthy

FSHD1

FSHD2

Epigenetically repressive

Epigenetically amenable

\[ \bullet = \text{Hypermethylated CpGs more heterochromatic} \]

\[ \bigcirc = \text{Hypomethylated CpGs more euchromatic} \]

A putatively pathogenic FSHD1 deletion shows very low penetrance

Healthy

Unaffected

FSHD1A

The deletion itself is not pathogenic
The 4qA sub-telomere is not pathogenic
The genetics are permissive, not pathogenic


~1% of population

~1:7-14,000
FSHD is linked to the A type subtelomere and the epigenetic status of the 4q35 D4Z4 repeat.

Healthy

Epigenetically repressive

- Hypermethylated CpGs
  more heterochromatic

FSHD1

Epigenetically amenable

- Hypomethylated CpGs
  more euchromatic

FSHD2
Wellstone family cohorts of muscle biopsies and myogenic cell cultures from FSHD1-affected and 1st degree relatives

Subjects screened for FSHD clinically and genetically (Blood)

Genotyping (Iowa Wellstone)

Cell Culture (UMMS)

Biopsies (KKI-JHU) (Wagner)

Animal models (Emerson, Wagner & Kunkel)

mRNA & protein analysis (Emerson, King & Kunkel)

Genetic analysis (Emerson, King, Kunkel, Wagner & Tupler)

Epigenetic analysis (Jones and Jones)

Deltoid – Expect less pathology

Biceps – Expect more pathology
The pathogenic *DUX4* gene is epigenetically “ON” in FSHD1 affected subjects

<table>
<thead>
<tr>
<th></th>
<th>A - FSHD1 affected</th>
<th>U - Healthy Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family 03</td>
<td></td>
<td>Family 03</td>
</tr>
<tr>
<td>Unmethylated</td>
<td>5.7%</td>
<td>72.7%</td>
</tr>
<tr>
<td>Methylated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family 09</td>
<td></td>
<td>Family 09</td>
</tr>
<tr>
<td>Unmethylated</td>
<td>6.4%</td>
<td>72.0%</td>
</tr>
<tr>
<td>Methylated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family 17</td>
<td></td>
<td>Family 17</td>
</tr>
<tr>
<td>Unmethylated</td>
<td>12.8%</td>
<td>71.4%</td>
</tr>
<tr>
<td>Methylated</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Epigenetically amenable**

- Unmethylated CpG

**Epigenetically repressive**

- Methylated CpG

T. Jones *et al.* (2014)
FSHD1-asymptomatic subjects are epigenetically more OFF than FSHD1-affected subjects

T. Jones et al. (2014)
DNA methylation profiles of asymptomatic subjects show intermediate levels of DNA methylation

From families with Asymptomatic carriers

Healthy
FSHD1 asymptomatic
FSHD1 affected

T. Jones et al. (2014)
The overall chromatin state of asymptomatic subjects is more epigenetically stable and refractory to gene expression.

Healthy

FSHD1

Asymptomatic

FSHD2

= More repressed chromatin

= More relaxed chromatin

= Mixture of chromatin states

= Hypomethylated CpGs

= Hypermethylated CpGs

= Intermediate methylation status

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

What are the implications for therapeutic development?
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
Dynamic/reversible → Druggable
Responsive
Numerous therapeutic targets for FSHD

Himeda et al. (2014) Antiox Redox Signaling
Antisense targeting of the cytoplasmic \textit{DUX4} mRNA; blocking DUX4 protein function

Himeda \textit{et al.} (2014) \textit{Antiox Redox Signaling}
Epigenetic regulation of \textit{D4Z4/DUX4} is a viable therapeutic target for FSHD

Himeda \textit{et al.} (2014) \textit{Antiox Redox Signaling}
Drug Therapies Can Also Target Epigenetics to Treat Disease Directly and Specifically

**Clinical Trials**

- New classes of epigenetic drugs target individuals with specific genetic defects in their tumors; toxicity is minimized
  - Targeting: HMTs (EZH2, LSD1, IDH1&2)
- Large area of drug development
  - Epizyme, Inc., Constellation Pharmaceuticals, EpTherapeutics, Agios Therapeutics, GSK, AstraZeneca, Novartis, among others
- Challenge: Identify subset of patients that can benefit from the treatments

**Pre-Clinical Development**

"Almost every big pharmaceutical company has a robust program in epigenetics. It’s quite a change in the past few years."

Yang Shi - JNCI, 2012
Summary

- Epigenetic regulation is context dependent, sequence independent; stable, heritable yet reversible

- FSHD is an epigenetic disease
  Overall epigenetic status of the D4Z4 correlates with clinical FSHD

- Small but significant epigenetic differences between being asymptomatic and FSHD-affected

- Epigenetic status of the D4Z4 is a viable FSHD therapeutic target

- Many drugs targeting epigenetic modifications are being developed for many diseases and may be applicable to FSHD
Individual epigenetic status of the FSHD-associated D4Z4 macrosatellite correlates with disease

At University of Massachusetts Medical School (and formerly BBRI)
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