Small RNAs Can Pack a Punch in Facioscapulohumeral Dystrophy

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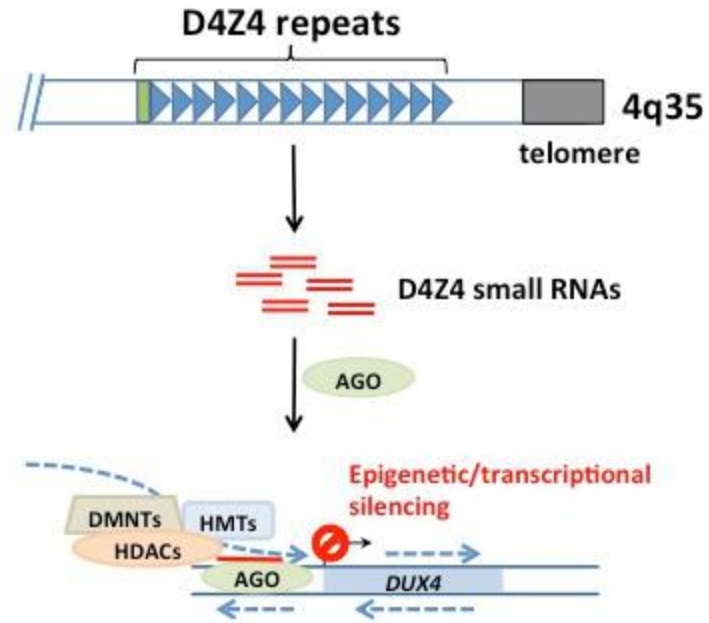

RNA-mediated epigenetic gene silencing of the D4Z4 repeats and the DUX4 gene embedded in the repeat.

Image provided by Dr. Filippova

Facioscapulohumeral dystrophy (FSHD) is a neuromuscular disorder characterized by progressive weakness of skeletal muscle, most noticeably in muscles of the face (facio), back (scapula) and upper arms (humeral), and is estimated to affect almost one million people worldwide. FSHD is thought to be caused by mis-expression of DUX4, a homeobox transcription factor that is normally only expressed in testes but is misexpressed in FSHD skeletal muscle cells. The *DUX4* gene resides within a macrosatellite repeat known as D4Z4, and the size of the D4Z4 array correlates with FSHD severity. For example, unaffected individuals have between 11 and 100 units of the array, whereas contraction of the array to between 1 and 10 units is linked to FSHD. However, the epigenetic mechanisms that repress DUX4 expression in normal muscle cells remain to be completely elucidated. A new Fred Hutch study by the Tapscott Laboratory (Human Biology and Clinical Research Divisions), led by Drs. Jong-Won Lim, Laurie Snider and Gala Filippova, and published in *Human Molecular Genetics*, showed that the endogenous RNAi pathway is required to silence the D4Z4 repeat array and DUX4 expression and that exogenous small interfering RNAs (siRNAs) that mimic endogenous D4Z4 small RNAs enhance epigenetic silencing of the D4Z4 array. "Our work

suggested a potential mechanism by which the D4Z4 repeat (and the *DUX4* gene imbedded in the repeat) is silenced by small RNAs generated from D4Z4 in the nonpathogenic condition. We expect therapeutic targeting strategies based on this mechanism would be effective for epigenetic silencing of *DUX4*, the FSHD causing gene, in FSHD condition," said Dr. Lim.

The authors had previously identified bidirectional transcripts and small RNAs throughout the D4Z4 region. To determine if such RNAs could modulate DUX4 expression, the researchers synthesized siRNAs that targeted not only the coding region of *DUX4*, but also the upstream non-coding region, often referred to as the promoter. Remarkably, the promoter siRNAs could suppress DUX4 expression as potently as the coding region siRNAs, albeit with delayed kinetics. This delayed effect is consistent with a model in which promoter siRNAs repress DUX4 transcription, whereas coding region siRNAs degrade the DUX4 mRNA directly. Because siRNA-dependent transcriptional silencing is known to involve Argonaute (AGO) proteins and dimethylation of Histone H3 on lysine 9 (H3K9me2), the authors examined both AGO recruitment and H3K9 methylation status by chromatin immunoprecipitation. This analysis revealed that promoter siRNAs increase the amount of AGO2 and H3K9me2 at the D4Z4 repeats.

To further probe the role of the RNAi pathway in the epigenetic silencing of DUX4, the authors knocked down DICER1, AGO1 and AGO2 in control myoblasts derived from unaffected individuals that had 13 D4Z4 repeats, which is in the low range of the non-FSHD population. Strikingly, the authors observed de-repression of both DUX4 and DUX4 target genes in cells that contained 13 D4Z4 units. In contrast, knockdown of RNAi components in control cells obtained from unaffected individuals that contained 74 D4Z4 repeats did not result in DUX4 de-repression. Furthermore, knocking down AGO2 prevented promoter siRNAs from suppressing DUX4 expression in FSHD cells. Importantly, targeting non-coding RNAs upstream of *DUX4* for degradation was not sufficient to de-repress DUX4, consistent with the proposed RNAi mechanism.

"Although still not widely accepted, this evolutionarily conserved RNA-mediated silencing mechanism clearly plays an important role in keeping repeats and genes imbedded in the repeats under control in both normal development and disease,such as FSHD" said Dr. Filippova. Overall, this study demonstrated the role of the endogenous RNAi pathway in epigenetic repression of DUX4, and suggests that enhancing the activity of this pathway with exogenous siRNAs could be a valid therapeutic approach to silence DUX4 in FSHD.

Lim J-W, Snider L, Yao Z, Tawil R, van der Maarel SM, Rigo F, Bennett CF, Filippova GN, Tapscott SJ. 2015. DICER/AGO-dependent epigenetic silencing of D4Z4 repeats enhanced by exogenous

siRNA suggests mechanisms and therapies for FSHD. Hum Mol Genet. pii: ddv206. DOI: 10.1093/hmg/ddv206. Epub ahead of print.

See also:

Hewitt JE. 2015. Loss of epigenetic silencing of the DUX4 transcription factor gene in facioscapulohumeral muscular dystrophy. *Hum Mol Genet*. pii: ddv237. Epub ahead of print.

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