FSHD Therapeutic Approaches: Gene therapy for FSHD

FSHD is a pathogenic gain-of-function disease amenable to multiple gene therapy approaches.

What is gene therapy?

"Gene therapy is not the same as taking a pill from the pharmacy. It's more like getting an organ transplant."
Mildred Cho, PhD. Member NIH Recombinant DNA Advisory Committee and Professor of Pediatrics & Medicine, Stanford University
https://stanmed.stanford.edu/2018winter/CRISPR-for-gene-editing-is-revolutionary-but-it-comes-with-risks.html

Gene therapy approaches use the delivery of exogenous genetic material (typically DNA) to change the course of a disease. Many aim to treat (or alter) the disease at the genetic level by adding new genes or editing existing genes (correction or disruption); however, some approaches may target a pathogenic mRNA (e.g., RNAi/miRNA or CRISPR-Cas13 gene therapies).

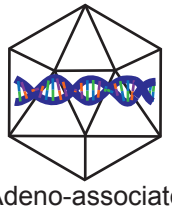
There are, of course, risks to gene therapy, including:
- → Immune reactions to the new or fixed protein AND/OR to the delivery (usually a virus).
- → Adverse off-target effects if genome insertion or editing is in the wrong place. This can include oncogenesis (cancer) and aberrant gene activation or repression.

However, gene therapy does not necessarily mean inserting DNA into or altering someone's genome!

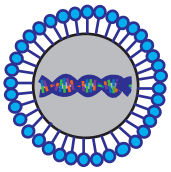
How is a gene therapy delivered?

***In vivo* administration (*i.e.*, directly into your body).**

Viral delivery is a common mechanism for getting the gene therapy to the target organs or to treat systemically, as one would for FSHD.



AAV = Adeno-associated virus (Does not integrate into your genome but maintains lifetime expresson of the gene.)



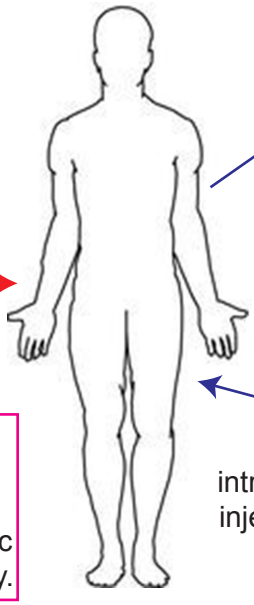
Lipid nanoparticles (For integration of the DNA into your genome.)

FSHD gene therapy will utilize AAV delivery.

Intravenous injection (systemic)

Intramuscular injection (local)

Both delivery modalities can be engineered to target specific cells in the body.



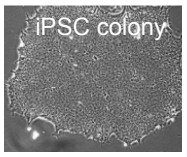
Gene therapy combined with cell therapy

***Ex vivo* administration (*i.e.*, treat in the lab).**

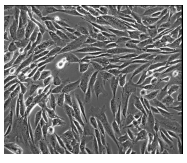
Many techniques for "fixing" cells in the lab; the hard part for FSHD will be getting them to where they are needed in the body.

Skin biopsy to isolate fibroblast cells to generate iPSCs (your own stem cells).

DNA or RNA



Correct defect (mutation) or, in FSHD, eliminate *DUX4,* and select for specificity.



Generate muscle precursor cells.

Local intramuscular injections for FSHD.

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Follistatin expression to allow for hypertrophic muscle growth -does not affect *DUX4* expression levels- delivered by AAV, from the Harper Lab (Giesige *et al*., 2018).

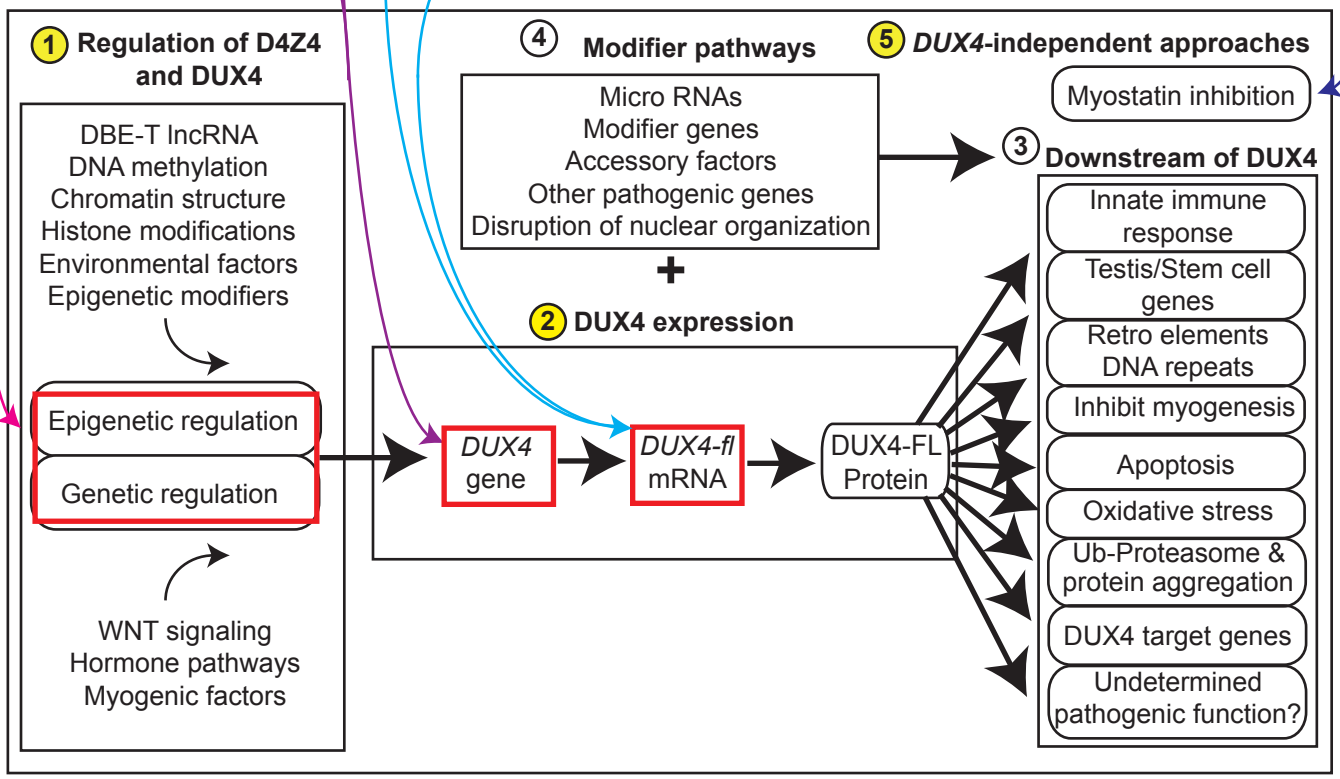
CRISPR-inhibition using AAV-delivered CRISPRi approach to shut off the *DUX4* gene, from the Jones lab (Himeda *et al*., 2016 and 2021).

CRISPR base-editing to destroy the *DUX4* PAS or alter DUX4 mRNA splicing to prevent DUX4 protein translation, from the van der Maarel lab (Sikrova *et al*., 2021).

CRISPR-editing (cutting) to destroy the DUX4 ORF (coding sequence) or PAS to prevent DUX4 protein translation; not likely viable (Joubert *et al*., 2020).

miDUX4.405, an AAV-delivered RNAi approach to knockdown the DUX4 mRNA, from the Harper lab (Wallace *et al*., 2012).

CRISPR-Cas13b (AAV-delivered CRISPR approach to knockdown the DUX4 mRNA, from the Harper lab).



Gene therapy targets in FSHD include the regulation of 1) *DUX4* gene expression using CRISPR-inhibition or CRISPR-activation, 2) the DUX4 mRNA, including antisense miRNA and CRISPR editing of the *DUX4* PAS or mRNA splicing sites, and 5) DUX4-independent amelioration of muscle weakness.