EPI-321: A Promising Gene Therapy for Facioscapulohumeral Muscular Dystrophy (FSHD) Targeting D4Z4 Epigenome

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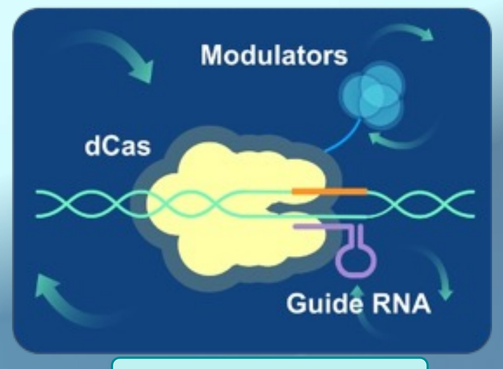
Epic Bio - Who We Are?

- CRISPR3.0 Epigenome Engineering Platform Biotech
- Proprietary Gene Expression Modulation System (GEMS) Platform
- GEMS can modulate single or multiple genes persistently or transiently facilitating broad pipelines
- Compact and interchangeable components that can support regulation of single and multiple genes *in vivo* (AAV or LNP) and *ex vivo* (Lentivirus and Retrovirus
- Exclusive License to CasMINI- smallest known Cas effector shown to function in human cells



Overview of GEMS Platform

ABSTRACT

Facioscapulohumeral muscular dystrophy (FSHD) affects 800,000 globally with no cure available, current therapies only manage symptoms. Disease-causing DUX4 protein expression in muscle leads to progressive muscle wasting through activation of apoptotic and other pathways. *DUX4* is encoded in the distal region of *4q35* chromosome from D4Z4 microsatellite array, which is hypomethylated in FSHD leading to *DUX4* expression.

At Epic Bio, we leveraged our proprietary GEMS platform to develop EPI-321, a treatment for FSHD that targets the D4Z4 epigenome and suppresses DUX4 expression permanently. EPI-321 is an AAV serotype rh74 vector with a catalytically inactive Cas protein fused to gene-suppressing modulators and a gRNA targeting D4Z4. EPI-321 showed no off-target to any known human protein coding gene *in silico*. We showed that EPI-321 robustly suppress DUX4 and downstream genes in patient derived myoblasts *in vitro,* irrespective D4Z4 repeat length. Functionally, *in vitro* treatment of FSHD myoblasts with EPI-321 decreased rate of apoptotic nuclei. Further, we showed robust delivery and expression of EPI-321 in the humanized muscle tissue *in vivo* following a single intravenous dose in mice. In addition to decreasing the DUX4 pathway, EPI-321 was able to decreased TUNEL+ cells after 4 weeks of treatment and showed dose dependent decrease in SLC34A2 protein - a DUX4 biomarker. We also show that EPI-321 improved muscle twitch and tetanus forces upon treatment in 3D organoid model of FSHD tissue. Importantly, EPI-321 in mice and NHP demonstrated no signs of toxicity, with no abnormal clinical, histopathological, or blood chemistry responses, indicating the safety of the treatment(data not shown).

Our findings support EPI-321 as a potential gene therapy for FSHD, with IND submission planned for 2023 and first-in-human trials in 2024.

BACKGROUND

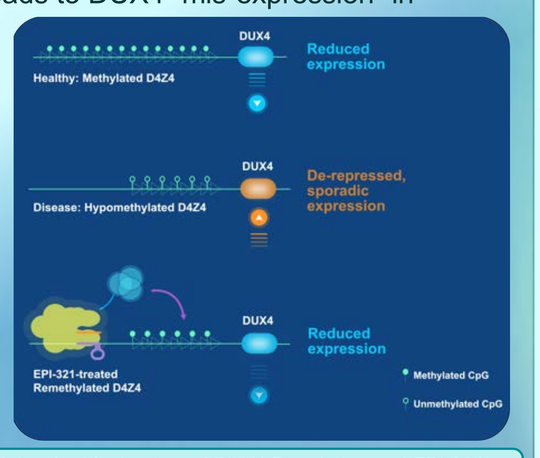
- Facioscapulohumeral Muscular Dystrophy (FSHD) is a debilitating genetic disorder leading to progressive muscle degeneration.
- Progressive weakness resulting in loss of movement of the face and loss of extremity function and mobility.
- Muscle degeneration pathology due to increased muscle cell death.
- Epigenetic rare disease due to loss of methylation that leads to DUX4 "mis-expression" in skeletal muscle.

Epidemiology

- US Population: 16,000-38,000
- Global Population: 300,000-780,000
- One of the Most Common Adult Muscular Dystrophy

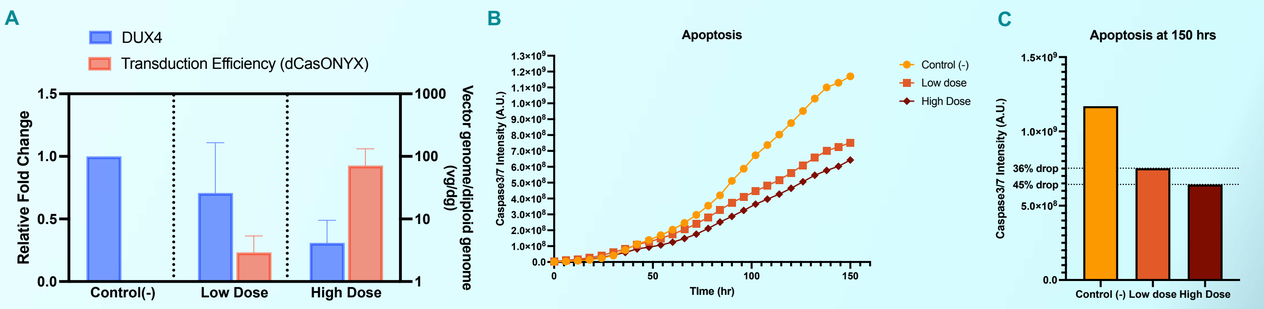
Standard-of-Care

- No disease-modifying drug available
- Exercise has been shown to reduce chronic fatigue and decelerate fatty infiltration of muscle in FSHD
- Surgery to treat scapulothoracic fusion



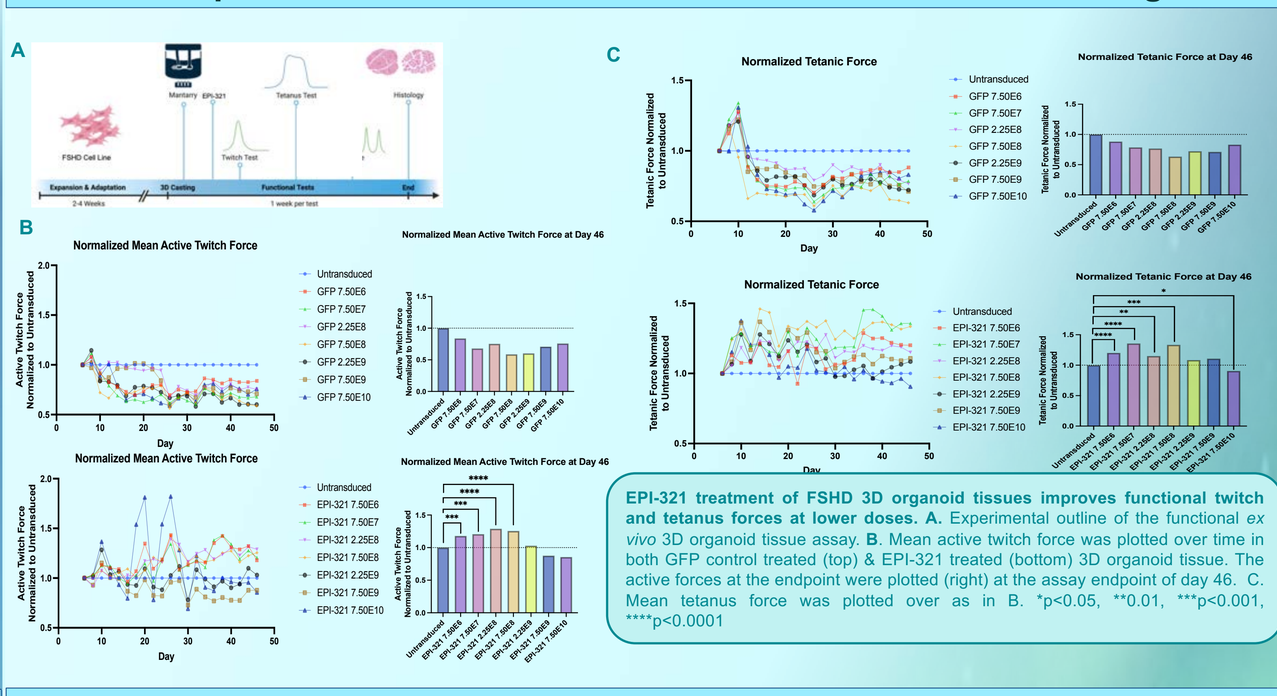
Molecular Mechanism of DUX4 Regulation and EPI-321 Approach to Treat FSHD

EPI-321 Shows Dose Response In FSHD Patients-Derived Primary Myoblasts



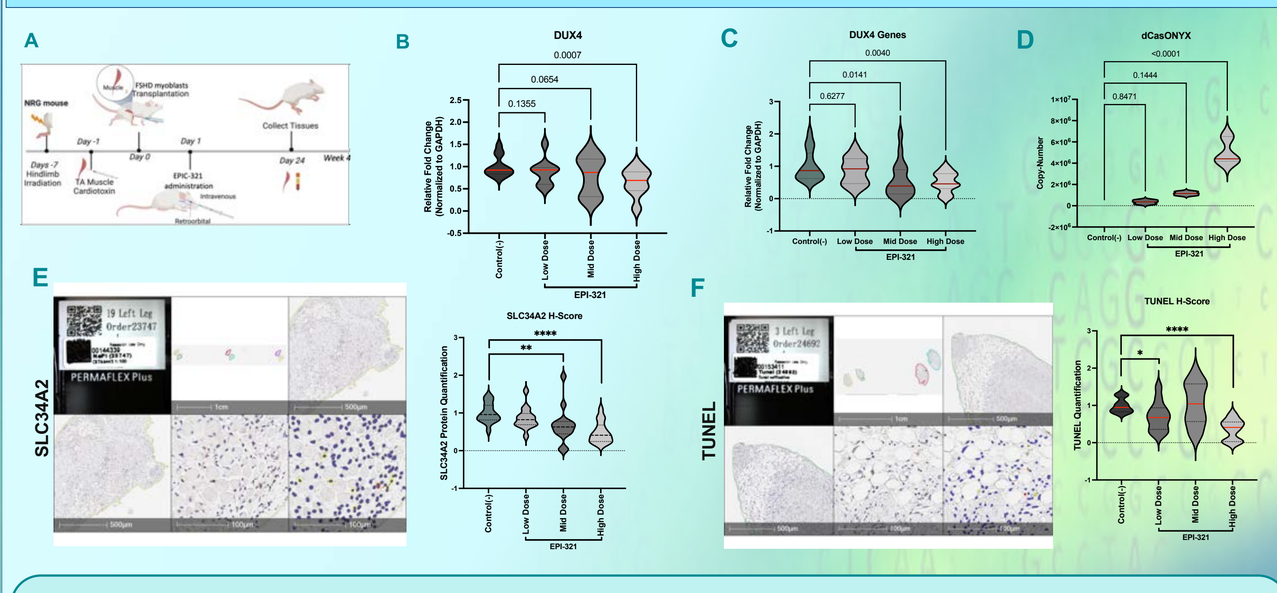
EPI-321 represses *DUX4* and rescues apoptotic phenotype in FSHD primary myoblasts. A-D. mRNA expression of DUX4 and EPI-321 cargo, dCasONYX at low and high dose in two different primary myoblasts. B. Caspase 3/7 stained live cell imaging analysis in primary myoblasts at low and high doses of EPI-321. The assay was performed using CellCyte Live cell imaging for up to 7 days of differentiation. C. The bar graph shows normalized intensity of total Caspase3/7 signal at the endpoint.

EPI-321 Improves Twitch and Tetanus Forces In 3D *ex vivo* FSHD Organoid



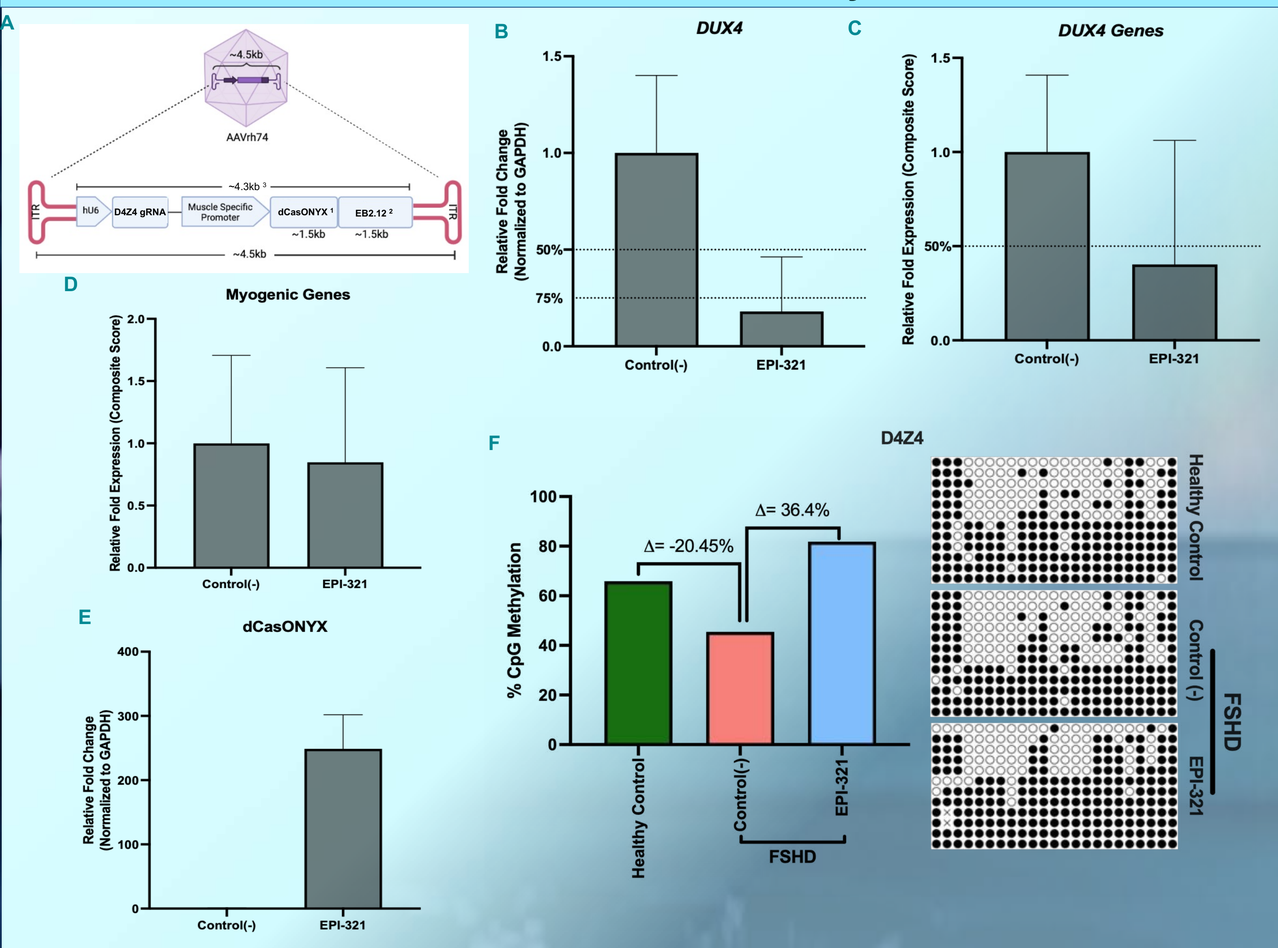
EPI-321 treatment of FSHD 3D organoid tissues improves functional twitch and tetanus forces at lower doses. A. Experimental outline of the functional *ex vivo* 3D organoid tissue assay. **B**. Mean active twitch force was plotted over time in both GFP control treated (top) & EPI-321 treated (bottom) 3D organoid tissue. The active forces at the endpoint were plotted (right) at the assay endpoint of day 46. C. Mean tetanus force was plotted over as in B. *p<0.05, **0.01, ***p<0.001, ****p<0.0001

EPI-321 Suppresses *DUX4* & DUX4-genes, And Also Improves FSHD Muscle Cell Survival In Humanized Mice



EPI-321 represses DUX4 and improves DUX4-associated phenotypes in humanized mice. A. Schematic showing *in vivo* experimental outline using humanized mice model. B. mRNA expression of *DUX4* in humanized TA muscle tissue of mice that were treated at three different doses of EPI-321. C. Animal tissue as in B were assayed for DUX4 pathway genes *ZSCAN4, MBD3L2, LEUTX, TRIM43*. Pathway genes expression were plotted as composite score. D. Biodistribution of EPI-321 assayed by qPCR estimate of EPI-321 cargo in tissues as in B. E. Tissues in B. were stained for SLC34A2 protein- a biomarker for DUX4 activity. Images were quantified and plotted (right). F. Tissues in B. were stained for TUNEL- a marker for DNA damage and apoptosis. Images were quantified and plotted (right).

EPI-321 Represses DUX4 & Downstream Genes Through Remethylation of D4Z4 In FSHD Patients-Derived Myoblasts



EPI-321 restores D4Z4 epigenome through DNA re-methylation in patient derived FSHD myoblasts. A. EPI-321 AAV Design. [1]**Safety:** EPI-321 utilizes a proprietary library of compact nuclease-dead versions of CRISPR (dCas), resulting in **NO DNA cuts.** [2]**Precision:** EPI-321 controls expression of the endogenous gene through methylation of the target sequence. [3]**Delivery:** EPI-321 is ultracompact, allowing it to be packaged it into AAVrh74. **B-E.** mRNA expression of DUX4 (B), geometric mean of DUX4 genes namely MBD3L2, LEUTX, ZSCAN4, TRIM43, TRIM48 & RFPL2 represented as composite score (C), geometric mean of myogenic genes namely MYOG & MYH2 presented as composite score (D) and dCasONYX (E). **F**. Percent of CpG methylation at EPI-321 targeting D4Z4 locus in healthy sibling control, FSHD patient derived myoblasts treated with control AAV and EPI-321 (Left panel). Median % CpG methylation assayed by Enzymatic conversion of DNA followed by sanger sequencing of the target loci. Sequences are analyzed using QUMA tool with default parameters. A representative lollipops plot is shown (Right panel).

CONCLUSION

- Epic Bio's GEMS screening platform identifies highly efficient effector-modulator combination suitable for treating genetic disease with unmet need like FSHD.
- EPI-321 is a compact AAV product that utilizes hypercompact nuclease-dead Cas molecule and modulates endogenous gene through methylation of target sequence.
- EPI-321 represses DUX4 target locus and decreases expression of downstream DUX4-pathway genes expression both *in vitro* FSHD patient derived myoblasts and humanized *in vivo* mice model.
- EPI-321 improves functional twitch and tetanus forces in ex vivo 3D organoid tissues from FSHD myoblast.
- EPI-321 also rescues the apoptosis level *in vitro* in patient derived myoblast and improve FSHD myoblasts survival in humanized mice model *in vivo*.
- EPI-321 has clean safety profiles in both immunocompetent mice and NHP that shows no signs of toxicity or severe immune response to EPI-321(data not shown).

ACKNOWLEDGEMENT

SOLVE FSHD

UMass Chan MEDICAL SCHOOL
Wellstone Center of FSHD

University of Rochester Medical Center
FSHD Research Center

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