PROJECT

Supervisor: __Giovanna Musco_____

Title: *Rational design of drug-like peptides blocking aberrant DUX4 in FSHD* _____

Curriculum: _____ Biologia Cellulare e Molecolare

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Link to the personal page of the University or relevant hospital site website: https://research.hsr.it/en/divisions/genetics-and-cell-biology/biomolecular-nuclear-magnetic-resonance/giovanna-musco.htm

Description of the Project (max 3,000 characters including spaces)

Background/gap of knowledge Aberrant activation in skeletal muscles of the transcription factor DUX4 and of the related pathways is associated to Facioscapulohumeral muscular dystrophy (FSHD), thus inhibition of DUX4 transcriptional activity constitutes a therapeutic opportunity against this severe genetic muscle disorder. Our collaborator Davide Gabellini at IRCCS San Raffaele has unveiled that MATR3 functions as a natural inhibitor of DUX4. Specifically, a fragment of MATR3 directly binds to DUX4 DNA-binding domains (DUX4dbd), preventing the activation of DUX4 target genes (1). As a result, myogenic and apoptosis defects observed in FSHD muscle cells are rescued. These data hold promises for the development of innovative MATR3-based therapeutics (e.g. mimicking peptides mPeps) able to recapitulate the interfering activity of MATR3, herewith blocking the detrimental function of DUX4 in FSHD

Rationale and hypothesis

The central hypothesis of this project posits that leveraging the MATR3-DUX4 interaction could inform the development of targeted therapies for FSHD, offering a natural and safe template for

designing drug-like molecules that target the DUX4 protein to selectively inhibit both its aberrant expression and its toxic activity

Objectives and specific aims The main goal of this proposal is the rational design of MATR3-based mPeps inhibiting aberrant DUX4 in FSHD. To this aim we will:

Structurally and thermodynamically characterize MATR3_FRAG/DUX4dbd complex by biophysical techniques (NMR, BLI, ITC, SAXS, AUC) and computational methods (docking, alphafold) herewith identifying key-residues for the interaction.

Rationally design MATR3-derived mPeps able to interact with DUX4dbd based on MATR3_FRAG/DUX4dbd complex

Evaluate MATR3-mPeps ability to inhibit DUX4 regulated gene expression and apoptosis in FSHD cellular models.

Expected outcomes

This project will not only offer molecular insights into the MATR3/DUX4 interaction, but will provide a proof of concept for a drug-like approach to inhibit DUX4 activity. As such it will pave the way for the development of MATR3-based peptidomimetics for the treatment of FSHD.

Skills that the student should acquire (max. 600 characters including spaces):

The student will learn: techniques for protein expression(in E. Coli) and purification, biophysical techniques(2) for structural and thermodynamic characterization of protein-ligand interactions (Nuclear Magnetic Resonance, Isothermal titration calorimetry, microscale thermophoresis; Analytical ultracentrifugation), Computational modelling; and biochemical and cellular assays for the cellular delivery of peptides, apopotosis assays, RNA extraction, reverse transcription, and quantitative real-time PCR

References (max. 15)

1. Runfola,V., Giambruno,R., Caronni,C., Pannese,M., Andolfo,A. and Gabellini,D. (2023) MATR3 is an endogenous inhibitor of DUX4 in FSHD muscular dystrophy. *Cell Rep*, **42**, 113120.
2. Mantonico MV, De Leo F, Quilici G, Colley SL, De Marchis F, Crippa M, Mezzapelle R, Schulte T, Zucchelli C, Pastorello, *et al.* (2024) The acidic intrinsically disordered region of the inflammatory mediator HMGB1 mediates fuzzy interactions with CXCL12. *Nat Commun*, **15**, 1–18.