PROGETTO

Supervisore::	Davide Gabellini
Titolo/*Title*:	Structure-function characterization of the DUX4-MATR3 complex
Curriculum:	Cellular and Molecular Biology
Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento:	https://research.hsr.it/en/divisions/genetics-and-cell-biology/gene-expression-regulation.html

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

FSHD is one of the most common neuromuscular disorders. The disease is caused by inappropriate gain of expression of the DUX4 gene, encoding for a transcription factor normally restricted to early embryonic development and silent in healthy skeletal muscle. DUX4 aberrant reactivation in FSHD interferes with myogenic differentiation and leads to apoptotic cell death resulting in muscle wasting [1].
We recently identified Matrin 3 (MATR3) as a natural inhibitor of DUX4 activity. We found that MATR3 binds directly to DUX4 DNA-binding domain and blocks activation of DUX4 target genes. In doing so, MATR3 rescues cell survival and myogenic differentiation of primary FSHD muscle cells. Importantly, we identified a small MATR3 fragment able to recapitulate the inhibitory activity of the full-length protein and show that its delivery to primary muscle cells is safe and effective [2].

Rationale and hypothesis

Other investigators are trying to develop ways to inhibit DUX4 expression or its toxic effects by focusing on small molecules compounds, gene therapy or antisense oligonucleotides. The novelty and added value of our project is that it is based on a physiological inhibitor of DUX4. For the first time, we have identified a protein able to bind DUX4 and block its activity. Building on this, we propose to develop a MATR3-based proteolysis targeting chimera (PROTAC) combining the ability to block both DUX4 expression and activity within a single molecule. Peptides are highly selective and efficacious and, at the same time, relatively safe and well-tolerated. Moreover, PROTACs have a catalytic mechanism of action, leading to increased durability of response and lowered susceptibility to resistance mutations.

Objectives and specific aims

Based on our results, we plan to perform structure-function characterization studies to develop drug-like MATR3-based peptidomimetics combining the ability to block both DUX4 expression and activity within a single molecule. To this purpose, the student will pursue the following Specific Aims:

1. To characterize the DUX4-MATR3 complex
Our preliminary data locate the MATR3 region binding to DUX4 within a small peptide. In collaboration with the Biomolecular NMR Unit, we will perform a detailed structural and biophysical characterization of the DUX4/MATR3 interaction to allow identifying the key MATR3 residues involved in DUX4 binding and the structure of MATR3 once bound to DUX4. This information will guide the rational design of MATR3 peptidomimetics which will be tested in Aim 2.

2. To test safety and efficacy of MATR3-based molecules in cellular and animal models of FSHD
We developed an efficient method to deliver peptides to the cell nucleus of muscle cells. Using the information from Aim 1, we will develop MATR3-based molecules combining the ability to inhibit and degrade DUX4. Safety and efficacy of the molecules will be tested in cellular and animal models of FSHD.

Expected outcomes

This project will provide a molecular understanding of MATR3 mechanism of action and a proof of concept for a drug-like approach to inhibit DUX4 for the treatment of FSHD.

Competenze che deve acquisire lo studente (Max 600 caratteri spazi inclusi):

The project entails a broad range of approaches, including biochemistry, gene expression profiling, next generation sequencing, cell biology, flow cytometry, immunoassays and animal models of disease. The student will learn how to critically evaluate the literature, design experiments, discuss the obtained results and assess their biological relevance. Training will also concern how to present scientific results at scientific meetings and write manuscripts or funding applications.

Bibliografia (max. 15)

1. Mocciaro, E., Runfola, V., Ghezzi, P., Pannese, M. & Gabellini, D. DUX4 Role in Normal Physiology and in FSHD Muscular Dystrophy. Cells 10, (2021).
2. Runfola, V. et al. MATR3 is an endogenous inhibitor of DUX4 in FSHD muscular dystrophy. Cell Rep 42, 113120 (2023).