Al collegio docenti del Dottorato in Medicina Molecolare

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Attività scientifica svolta nel I anno di Dottorato - Anno Accademico 2022/2023

Introduction

Congenital myopathies are an expanding group of genetic disorders affecting skeletal muscle. One of the main forms of congenital myopathies is **Central Core Disease** (CCD) typically presents in infancy where it manifests with hypotonia and motor developmental delay and is characterized by predominantly proximal weakness pronounced in the hip girdle. From the molecular perspective CCD is characterized by dominant/recessive mutations in RYR1 gene resulting in alteration of calcium homeostasis. Today, there are no therapies available for CCD, but the study conducted by *Alexander et al*. has demonstrated that miR-486 is capable of improves muscular function in the mdx mouse model of muscular dystrophy. miR-486 is a short (20-24 nt) non-coding RNA sequences involved in post-transcriptional gene regulation by interfering on stability or translation of mRNAs and acts in different molecular pathways including the PTEN/AKT signaling pathway.

To investigate whether miRNA-486 may also play a protective/ therapeutic role in the CCD we have decided to develop an adenoviral vector capable of inducing an overexpression of miR-486, using a murine disease model IT-mice ^{I4895T} (Human ^{I4898T}). The proposed strategy involves the use of **AAV9-PrimiR-486** at various concentrations inoculated at the mouse TA (Tibialis Anterior) in order to identify the concentration of AAV9-PrimiR-486 that allows for the increase in miR-486 in WT mice.

Method

Injections of AAV9-PrimiR-486 at 4 different concentrations: $0.21x10^{11}$vg/muscle, $1x10^{11}$ vg/muscle, $2.5x10^{11}$vg/muscle, $5x10^{11}$ vg/muscle were administered into the TA (Tibialis Anterior) of WT mice. At 15 and 30 days post-injection, injected TA muscles were collected from each mouse, after which RNA was extracted and qPCR performed to assess miR-486 expression levels using Taq-Man probes. Additionally, adenoviral particle expression levels were analyzed through EGFP detection. Furthermore, qPCR analysis was used to compare the muscles injected with AAV9-primiR-486 to the contralateral TA injected with PBS, and with the gastrocnemius muscle of transgenic mice overexpressing miR-486

Results

qPCR analysis, using-EGFP as a target for detection of adenoviral infection, has shown that in both 15 and 30 days post-injection, the quantity of adenoviral particles in TA increases with higher concentrations of AAV9-PrimiR-486 injections. The data obtained from qPCR for miR-486 revealed a 3 to 5 fold increase in the expression of miR-486 after injection with AAV9-primiR-489 compared to the TA non-injected (CTRL-) at 15 days after the injection. Additional experiments are being performed to verify and consolidate these data.

PARTECIPAZIONE A CORSI

SOFT SKILLS:

- 1. Introductory concepts and requirements for the birth of a company, *Prof. Lorenzo Zanni*, 15/05/2023
- 2. The business model and university regulation on spin-offs and startups, *Dr. Niccolò Fiorini*, 16/05/2023
- Europrogettazione: programmi, progetti e rendicontazione, 18/05/2023
- 3. Innovation ecosystem in support of startups, *Dr. Francesco Maria Senatore*, 18/05/2023
- Science and Society: continuity and change , 29/05/2023
- PhD and then? Overview on career paths inside and outside the academia, *Prof. Gennaro Prota and Dr.ssa Aida Ribera Navarro*

LEZIONI PhD:

- How to study genetics in complex diseases, *Prof. Stefano Landi*, Università di Pisa, 19/01/2023-26/01/2023
- Microvesicles in cell-to-cell comunication, *Dr.ssa Laura Governini*, Università di Siena, 27/02/2023
- Biomarkers discovery in precision medicine, *Dr.ssa Alice Luddi*, Università di Siena, 27/02/2023

CONGRESSI:

- PhDay, Firenze, 17/01/2023 - Dottorato in Medicina Molecolare Esame Finale 35°ciclo, 17/05/2023
- XXI CONVENTION SCIENTIFICA DI FONDAZIONE TELETHON, Riva del Garda, 13-14-15 Marzo 2023

EVENTUALI SOGGIORNI IN ALTRI LABORATORI ITALIANI O ESTERI:

Currently at *Grenoble Institute of Neurosciences– Centre de Recherche U1216 - Inserm - Univ. Grenoble Alpes*