PROGETTO

Supervisore	Maurizio D'Antonio
Titolo/ *Title:*	Dissecting the molecular mechanisms of TDP43 cell specific toxicity in *in vivo* models
Curriculum:	NEN
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/biology-of-myelin.html

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

TAR DNA-binding protein-43 (TDP-43)-positive inclusions in the brain and spinal cord are the neuropathological hallmark of amyotrophic lateral sclerosis (ALS), found in >95% of patients [1]. The mechanism by which TDP-43 causes neurodegeneration is still unknown, with evidence supporting both a nuclear loss-of-function and a cytoplasmic toxic gain-of-function [1]. TDP-43 positive aggregates have been recently reported in motor nerve biopsies of ALS patients. Their appearance occurs before axonal degeneration, and a relevant proportion of ALS patients displays TDP-43 aggregates not only in the axon, but also in the myelinating Schwann cells, suggesting the possible contribution of non-cell autonomous effects [2, 3].

Rationale and hypothesis

This project stems from a strict collaboration between the group of Dr. D'Antonio and the group of Dr Angelo Quattrini, that will act as a co-supervisor. Together, we recently generated mice overexpressing human mutant TDP-43 (hTDP43 p.A382T, the most frequent TARDBP mutation in Italian ALS patients)) selectively in Schwann cells (SC) (by crossing with P0-Cre mice) [4], as well as in ChAT-positive motor neurons (MN) (by crossing the hTDP43 mice with ChAT-Cre mice), using a Cre-lox system. Both lines ($hTDP43^{A382T\text{-}SC\text{-}OE(het)}$ and $hTDP43^{A382T\text{-}MN\text{-}OE(het)}$ respectively) show modest levels of TDP-43 overexpression, roughly equivalent to one extra copy of mutant TDP-43, as measured by qRT-PCR. Behavior, electrophysiology, and pathology were assessed longitudinally in both lines. The low TDP-43 expression lines develop subtle or no motor phenotypes with only mild cellular and molecular changes during the animal's lifespan. Specifically, $hTDP43^{A382T\text{-}SC\text{-}OE(het)}$ mice, starting at 9-months, show signs of axonal degeneration

and regeneration, and a few demyelination events in both sciatic and femoral nerves, while hTDP43$^{A382T\text{-}MN\text{-}OE(het)}$ display signs of MN degeneration.

Thus, in this project, to facilitate the molecular analysis of these mice and shed light on the mechanisms leading to SC or MN pathology following TDP43 overexpression, we sought to elevate the gene dosage by generating homozygous lines. We reasoned that conversion of one line into homozygosity could accelerate the disease: the increased gene dosage should result in higher expression of the transgene compared with the hemizygous mice, causing a more severe and earlier phenotype.

Objectives and specific aims

As such, in this project we propose to: 1) analyze the recently generated hTDP43$^{A382T\text{-}SC\text{-}OE(homo)}$ longitudinally (from 3 to 9-months of age) for behaviour, neurophysiology, pathology and biochemistry. 2) generate and analyze longitudinally (from 3 to 9-months) the hTDP43$^{A382T\text{-}MN\text{-}OE(homo)}$ line for behaviour, neurophysiology, pathology and biochemistry .Furthermore, 3) to test whether the hTDP43$^{A382T\text{-}MN\text{-}OE}$ mice could convert hTDP43$^{A382T\text{-}SC\text{-}OE}$ mice from a mild to a severe phenotype, we will develop and analyze double-transgenic mice overexpressing the human mutant TDP-43 in both SC and MN, initially in heterozygosity, and if needed in homozygosity.

Expected outcomes

With this work we expect to dissect the cell specific molecular mechanisms underlying TDP43 toxicity in the PNS, and to identify potential targets for novel therapies.

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

General skills: Perform independent literature search, study planning, pose a research question/problem, examine the range of available modes of inquiry, identify the appropriate research mode and procedure, identify a data collection strategy, analyze and interpret data, draw conclusion from the data and write research paper.

Specific skills: molecular biology and biochemistry techniques (PCR, WB, real-time PCR, transcriptomic), animal colony generation and management, transgenic mice analysis, light, electron and confocal microscopy techniques.

Bibliografia (max. 15)

1] Hardiman O et al. Nat Rev Dis Primers. 2017; 3:17071

[2] Riva N et. al Brain 2022;145(1):276-284

[3] Gentile F et. al Front Neurosci 2019;13:601

[4] Feltri ML et al. Eur J Neurosci 1999;11:1577-86

[5] Soriano P. Nat Genet 1999;21:70-1

[6] Rossi J et al. Cell Metab 2011;13:195-204