**PROGETTO**

**Supervisore:** **Franca Codazzi**

Titolo/*Title*: **Monitoring the contribution of upper motor neurons in ALS progression and propagation**

*Curriculum*: Neuroscienze e Neurologia Sperimentale

Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento: https://www.unisr.it/en/docenti/c/codazzi-franca_

**Descrizione del progetto (max 3.000 caratteri spazi inclusi)**

**Background/gap of knowledge**

Amyotrophic lateral sclerosis (ALS) is a progressive, lethal neurodegenerative disease (Tapia, 2014) that becomes manifest as a focal damage that progressively spreads across the central and peripheral nervous system. While many studies have focused on the cell intrinsic mechanisms underlying degeneration of lower motor neurons (LMNs), the contribution of upper motor neurons (UMNs) and cortico-spinal connectivity are poorly defined.

**Rationale and hypothesis**

Aberrant crosstalk between the corticospinal tract and spinal cord may enhance the spread of LMN toxicity and degeneration. Indeed, both "dying-back" (from muscle to LMNs to UMNs) and "dying-forward" (from UMNs to LMNs to muscle) mechanisms have been postulated (Baker, 2014). Recent findings from studies conducted in SOD1(G86R) mice and with mutant TDP-43 expressed in UMNs (Marques, 2021; Tsuboguchi, 2023), support the notion of a cortical origin of the disease and suggest that the anterograde dissemination of neurotoxic signals across neural circuits could activate specific molecular pathways associated with neurodegeneration.

**Objectives and specific aims**

Aim 1: Monitoring ALS propagation through in vivo translatome profiling of LMNs in conditional TDP-43 mouse models.

We will establish an in vivo molecular profiling approach based on Translating Ribosome Affinity Purification (TRAP) (Heiman, 2014), to monitor translatome changes occurring over time in LMNs as a result of TDP-43 de-regulation/mutation in UMNs. A conditional allele of TDP-43 harboring an ALS-linked mutation (TDP-43(Q331K)), a well characterized mouse model of ALS; Arnold et al., 2013) will be combined with UMN-specific Cre and and with a TRAP-reporter active in LMNs. Differentially expressed genes (DEGs) will be validated by mRNA in situ hybridization in mouse sections. The corresponding proteins will be analyzed by WB and IHC and their role examined in neuronal models of TDP-43 proteinopathy (Pisciottani, 2023). Part of this work will be in collaboration with D. Bonanomi (OSR, Milan)

Aim 2: investigating UMN functional alterations.

To characterize TDP-43 dysfunction in UMNs, we will culture UMNs obtained from the TDP-43(Q331K) mouse model, as detailed in Pisciottani, 2023. UMN populations will be subjected to studies of neuronal physiology (e.g. analysis of calcium homeostasis, mitochondrial function, and antioxidant properties, etc.) and synaptic function (Pisciottani, 2023; Codazzi, 2016). Part of the studies will be carried out on UMNs grown on an open bioengineered platform (Hagemann, 2024) permitting the growth of long axons and the assembly of complex in vitro circuits. (collaboration A. Serio, King's College).

**Expected outcomes**

Overall, we expect to elucidate the role of the UMNs in the onset and progression of neurodegeneration in ALS and to address the unresolved issue of early events and spread of neurotoxicity between the central nervous system (CNS) and peripheral nervous system (PNS).

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

Animal husbandry; breeding strategies; in silico analysis of RNAseq results (under expert supervision); analysis of mRNA and protein expression in mouse tissues; culture of primary neurons; functional studies; Advanced microscopy techniques.

**Bibliografia** (max. 15)

Tapia R. Front Cell Neurosci. 2014. DOI: 10.3389/fncel.2014.00241

Baker MR. Nat Rev Neurol. 2014 Nov;10(11):660. doi: 10.1038/nrneurol

Marques, RF et al., Hum Mol Genet, 2020. DOI: 10.1093/hmg/ddaa140.

Tsuboguchi, S et al., Acta Neuropathol. 2023. doi: 10.1007/s00401-023-02615-8.

Heiman, M., et al., Nat Protoc, 2014. DOI: 10.1038/nprot.2014.085

Arnold ES et al., Proc Natl Acad Sci U S A. 2013 doi: 10.1073/pnas.1222809110.

Pisciottani et al., 2023 Front Cell Neurosci. doi: 10.3389/fncel.2023.1253543

Codazzi et al. Hum Mol Genet. 2016. doi: 10.1093/hmg/ddw308.

Hagemann et al., PLoS Biol. 2024. doi: 10.1371/journal.pbio.3002503.