PROGETTO

Supervisore:: _____Dario Bonanomi_____

Titolo/*Title*: Probing and targeting dysfunctional cell phenotypes in motor neuron disease

Curriculum: __ Neuroscience and Experimental Neurology

Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento: https://research.hsr.it/en/divisions/neuroscience/molecular-neurobiology.html

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge Motor neurons (MNs) of the spinal cord are selectively affected in Amyotrophic Lateral Sclerosis (ALS), a neurodegenerative disorder in which all voluntary movements are lost. ALS is one of several diseases in which MNs die with remarkable specificity. What makes these cells vulnerable is unclear. The pathogenetic process may initiate cell-autonomously (within MNs due to their intrinsic properties) or non-cell-autonomously, due to toxic signals from neighboring cells. It is thought that understanding the molecular underpinnings of MN susceptibility will open new therapeutic opportunities.

Rationale and hypothesis We propose that dysfunction in the vascular compartment of the spinal cord, where MN cell bodies are located, and peripheral nerves that contain their long axonal projections is a critical contributor to the selective demise of MNs in ALS. Toxic signals derived from either endothelial cells that form the inner lining of blood vessels or associated perivascular cells might directly affect MNs or exacerbate damage derived from abnormal processes intrinsic to MNs. The combined action of pathogenic signals derived from MNs themselves or nearby vessels is expected to trigger neurodegeneration.

Objectives and specific aims The overall goal is to test the contribution of vascular cells to ALS pathogenesis and identify molecular pathways responsible for aberrant neurovascular crosstalk that, we hypothesize, underlies spreading of neurodegeneration. Within this general framework, the lab has already collected promising data on changes in the perivascular niche that appears to be an early responder in ALS (4). These results will serve as a baseline for a candidate approach during the initial stage of the project.

The PhD project will study deregulated molecular signatures in perivascular cells of the nerves and spinal cords of ALS mouse models. After key pathogenic processes are identified, the student will engineer adenoviral vectors (AAV) to establish preclinical targeting strategies based on manipulation of these pathways in different cell types of the nervous system. The student will join the lab efforts to build a single-cell reference map of ALS nerves with scRNA-seq and spatial transcriptomics, and will use histological assays to validate cellular and molecular changes predicted in silico. AAV vectors targeting candidate genes and, if possible, compound-based treatments will be tested in disease mouse models.

Expected outcomes Besides advancing the fundamental understanding of still elusive pathogenetic mechanisms, this project will establish a baseline for preclinical studies and future therapeutic applications directed at reverting abnormal neurovascular signaling. The study will reveal key molecular targets and enable the design of tools to enhance neuroprotection. Viral vectors specific for the CNS endothelium will be engineered to implement gene therapy in preclinical models.

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

Mouse handling; design of mouse crosses; basic surgeries and tissue dissection; immunohistochemistry/histology; Tissue clearing and imaging; molecular approaches including cloning, preparation of RNA probes, cDNA library preparation; FACS sorting and basic analysis; preparation of viral particles for cell culture transduction and in vivo injection; image processing and quantitative analysis of imaging and gene expression data. Interaction with bioinformaticians will be encouraged to build skills in interpretation and analysis of computational data (programming skills are optional).

Bibliografia (max. 15)

1. Hardiman et al., Amyotrophic lateral sclerosis. Nat Rev Dis Primers 3, 17071 (2017). DOI: 10.1038/nrdp.2017.71

2. Iadecola C. The Neurovascular Unit Coming of Age: A Journey through Neurovascular Coupling in Health and Disease. Neuron. Sep 27 2017;96(1):17-42. https://doi.org10.1016/j.neuron.2017.07.030.

3. Zlokovic BV. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. Nat Rev Neurosci. Nov 3 2011;12(12):723-38. https://doi.org10.1038/nrn3114.

4. Garbuzova-Davis S, et al. Impaired blood-brain/spinal cord barrier in ALS patients. Brain Res. Aug 21 2012;1469:114-28. https://doi.org10.1016/j.brainres.2012.05.056.

5. Manberg A, et al. Altered perivascular fibroblast activity precedes ALS disease onset. Nat Med. Apr 2021;27(4):640-646. https://doi.org10.1038/s41591-021-01295-9.

6. Riva et al. Phosphorylated TDP-43 aggregates in peripheral motor nerves of patients with amyotrophic lateral sclerosis. Brain. 2022;145(1):276-284. https://doi.org10.1093/brain/awab285.

7. Krolak T, et al. A High-Efficiency AAV for Endothelial Cell Transduction Throughout the Central Nervous System. Nat Cardiovasc Res. Apr 2022;1(4):389-400. https://doi.org10.1038/s44161-022-00046-4.