PROGETTO

Supervisore:: Jean-Michel Cioni_____

Titolo/*Title*: Unravelling the pathogenic processes behind Charcot-Marie-Tooth Type 2B disease.

Curriculum: ___NEN_____

Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento: __ https://research.hsr.it/en/divisions/neuroscience/rna-biology-neuron/jean-michel-cioni.html_____

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

Charcot-Marie-Tooth (CMT) disease is a clinically and genetically heterogenous group of hereditary neuropathies with a prevalence of around 1/2500 people. CMT has been divided in various subtypes (e.g. CMT1-6, CMTX) and a high number of mutations (around a 1000) in more than 80 genes have been characterized as causative. These disorders primarily affect the sensory and/or motor peripheral nervous system, leading to distal sensory loss and muscle weakness and atrophy. CMT type 2B (CMT2B) is an autosomal dominant axonal form of the disorder, mostly affecting sensory neurons, and with an onset usually in the second or third decade of life. So far, 6 missense mutations (p.K126R, p.L129F, p.K157N, p.N161T/I, p.V162M) in *RAB7A* gene have been described as causative for CMT2B. **At present, no efficient treatment exists to prevent, slow down or reverse the symptoms in patients with this, or any of the others, CMT neuropathy.**

Rationale and hypothesis

While the genetic cause of CMT2B disease has been identified, and some pathological processes have been suggested to play a role in the disease, to our knowledge no preclinical trial has never been attempted. A clear limitation is the absence of a suitable model for *in vivo* analysis, a mandatory requirement to better define the pathology behind the disorder and test new therapeutic approaches. To reply to this need, we recently generated a mouse model in which the CMT2B-associated N161 > T mutation was achieved in *Rab7a* gene (*Rab7a*^{N161T} mice). Here we propose to perform a comprehensive analysis of the pathological features that occur in this model that mimics the human disorder from a genetic perspective.

Objectives and specific aims

Interestingly, preliminary findings already identified some defects in lysosomal and mitochondrial dynamics in neurons carrying CMT2B Rab7a^{N161T} mutation, supporting the possibility that mitochondria represent interesting targets for potential therapies. In this proposal, we therefore propose to characterize our CMT2B-

Rab7a^{N161T} mouse model by i) determining the functional consequences of Rab7a^{N161T} mutation *in vivo,* ii) clearly define the alterations in endosomal and mitochondrial trafficking induced by *Rab7a^{N161T}* mutation in sensory axons, and iii) test drug-based approaches to counteract the observed phenotypes.

Expected outcomes

Altogether, our data will provide a clear picture of the *in vivo* consequences of *Rab7a^{N161T}* mutation on the peripheral nerve morphology and associated functions in mice. We will also characterize the alterations in time, providing important knowledge for potential therapeutic strategies. Our results will be essential to determine if the alterations observed in *Rab7a^{N161T}* mice mimic the pathology seen in CMT2B patients, and therefore to which extent it can be used for *in vivo* preclinical studies in the future.

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

The student will acquire:

Technical skills: Primary culture of neuronal cells, live-imaging approaches, immunocytochemistry, biochemical methods, in vivo analysis of nerve morphology, pharmacology treatments.

Image acquisition and analysis using specialized softwares (e.g. FIJI, NIS-Elements,…)

Data management and presentation (national and international meetings).

Bibliografia (max. 15)

Zhang, K., et al., Defective axonal transport of Rab7 GTPase results in dysregulated trophic signaling. J Neurosci, 2013. 33(17): p. 7451-62.

Spinosa, M.R., et al., Functional characterization of Rab7 mutant proteins associated with Charcot-Marie-Tooth type 2B disease. J Neurosci, 2008. 28(7): p. 1640-8.

Cisneros, J., et al., Mitochondria-lysosome contact site dynamics and misregulation in neurodegenerative diseases. Trends Neurosci, 2022. 45(4): p. 312-322.

Cioni, J.M., et al., Late Endosomes Act as mRNA Translation Platforms and Sustain Mitochondria in Axons. Cell, 2019. 176(1-2): p. 56-72 e15.

Wong, Y.C., W. Peng, and D. Krainc, Lysosomal Regulation of Inter-mitochondrial Contact Fate and Motility in Charcot-Marie-Tooth Type 2. Dev Cell, 2019. 50(3): p. 339-354 e4.