PROGETTO

Supervisore:: Jenny Sassone

Titolo/*Title*: INVESTIGATING THE ROLE OF NECROPTOSIS IN THE AXONAL DEGENERATION ASSOCIATED WITH PARKINSON'S DISEASE

Curriculum: NEN

Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento: https://www.unisr.it/docenti/s/sassone-pagano-jenny

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

Autosomal Recessive Juvenile Parkinsonism (ARJP) is a genetic form of Parkinson's disease (PD) caused by mutations in the gene *PRKN* (1) and characterized by dopamine (DA) neuron death in the substantia nigra. Evidence suggests that DA neuron death is preceded by axonal degeneration (2) but the molecular mechanism underlying axonal degeneration in ARJP are still unclear. Necroptosis is a programmed cell death mechanism regulated by the kinases RIPK1, RIPK3, and the downstream effector MLKL. Since evidence suggests that necroptosis is involved in axonal degeneration and DA neuron death (3–5), this project will test the hypothesis that necroptosis mediates axonal degeneration and DA neuron death in a mouse model of ARJP.

Rationale and hypothesis

We will test the hypothesis that necroptosis has a role in the axonal degeneration of DA neurons in the nigrostriatal tract of the mouse model *Prkn*R275W (https://biorxiv.org/cgi/content/short/2023.09.26.559326v1).

SA1. To investigate necroptosis markers in the nigrostriatal tract of *Prkn*R275W mice. Since previous evidence suggest a role of necroptosis in the DA neuron death (6), we will analyze *Prkn*R275W brains to detect necroptosis features.

SA2. To test the hypothesis that necroptosis inhibition prevents DA neuron loss in *Prkn*R275W mice. Due to the indispensable role of MLKL in executing necroptosis, we will evaluate whether genetic ablation of MLKL prevents *PRKN* related axonal damage and DA neuron.

Objectives and specific aims

SA1.1. Since MLKL oligomerization has a key role in executing necroptosis, we will analyze the oligomerization of MLKL by native PAGE gels in the brain tissues of WT and *Prkn*R275W mice.

SA1.2. Since necroptosis activation is mediated by necrosome formation (7), we will analyze the interaction between RIPK1, RIPK3 and MLKL in the brain tissues of WT and *Prkn*R275W mice by Proximity ligation assay.

SA1.3. Since necroptosis is associated with neuroinflammation (8) we will analyze a panel of neuroinflammation markers by RT-PCR in the brains of WT and *Prkn*R275W mice.

SA2.1. We will generate double transgenic MLKL KO/*Prkn*R275W mice. Since both MLKL KO and *Prkn*R275W mice are viable and fertile (9) we expect to obtain double transgenic mice at mendelian rate.

SA2.2. Double transgenic MLKL KO/*Prkn*R275W mice will be analyze for markers of axonal damage by analyzing the levels of neurofilaments (10,11).

SA2.3. Double transgenic MLKL KO/*Prkn*R275W mice will be analyzed for markers of neuroinflammation.

SA2.4. We will test the hypothesis that genetic ablation of MLKL prevents DA neuron loss. Double transgenic MLKL KO/*Prkn*R275W mice will be analyzed by unbiased sterological count of DA neurons.

Expected outcomes

SA1. Statistically significant data (SSD) about MLKL oligomerization, PLA analyses and neuroinflammation markers.

SA2. Generation of double transgenic MLKL KO/*Prkn*R275W mice. SSD about axonal damage, neuroinflammation and DA neuron number.

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

Management of wild-type and transgenic mouse colonies, immunofluorescence analyses, confocal microscopy, biochemical assays, stereological count of DA neurons.

The PhD student will learn to define a problem and identify possible causes and solutions, design and plan an experiment, identify goals and/or tasks to be accomplished and a realistic timeline for completion.

The PhD student will learn to conduct group discussions, present data at scientific meetings and organize and communicate ideas effectively in oral presentations.

Bibliografia (max. 15)

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12. Regoni, M., Cattaneo, S., Mercatelli, D., Novello, S., Passoni, A., Bagnati, R., Davoli, E., Croci, L., Consalez, G.G., Albanese, F., *et al.* (2020) Pharmacological antagonism of kainate receptor rescues dysfunction and loss of dopamine neurons in a mouse model of human parkin-induced toxicity. *Cell Death Dis.*, **11**, 963.
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