DOTTORATO DI RICERCA IN BIOLOGIA CELLULARE E DELLO SVILUPPO
40° Cycle

Project proposal for a PhD scholarship (with no financial support from Sapienza)

Title of the research: In-vitro studies of motor neuron degeneration mechanisms in Amyotrophic Lateral Sclerosis

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Summary

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting motor neurons, resulting in progressive muscle weakness and death by respiratory failure. Protein aggregation and neuroinflammation are central to the pathology of ALS. Due to its functions in autophagy (the main cellular process for removal of protein aggregates) and the innate immune response, the TANK-binding kinase 1 (TBK1), is a protein with key roles in ALS pathological processes. Exome sequencing studies identified loss of function mutations in the *TBK1* gene in sporadic and familial ALS patients. Interestingly, some ALS patients carry mutations in both *TBK1* and other known ALS-associated genes (such as *TARDBP* and *C9ORF72*), suggesting that *TBK1* might act as a disease modulating gene in these cases.
We have previously pioneered an oligogenic approach to study mechanisms of TBK1-dependent neurodegeneration. By combining *Tbk1* mutations with *Sod1* mutations in mice, we have demonstrated that loss of TBK1 function in motor neurons of the well-studied SOD1G93A mouse model of ALS impairs autophagy, increases SOD1 protein aggregates, and accelerates motor neuron death and muscle denervation. This results in early disease onset and decreased motor performance of SOD1 mice. These data suggest the hypothesis that loss of TBK1 function specifically in motor neurons may be responsible for the ALS phenotype in patients through disruption of autophagy and increased protein aggregation. We aim here to test this hypothesis by generating *TBK1*-CRISPR knock-out iPSCs and differentiating them into motor neurons. We will also test the hypothesis that TBK1 loss exacerbates neurodegeneration and protein aggregation in patients with TDP-43 and C9ORF72 mutations. We will use a multidisciplinary approach combining transcriptomics, phosphoproteomics, biochemistry and immunohistochemistry, to evaluate the effects of TBK1 loss on cell viability and protein aggregation in human motor neurons. The multi-omic data generated by the proposed project will unveil (*i*) intracellular pathways affected by loss of TBK1 in motor neurons; (*ii*) common and divergent mechanisms of motor neuron degeneration caused by TBK1 loss of function mutations in patients bearing other ALS-associated variants (*i.e.*, TDP-43 and C9ORF72). This project will provide the rationale for a novel multigenic approach to disease modelling in human cells and reveal new therapeutic targets to halt neurodegenerative processes caused by TBK1 loss.

Pertinent Publications of the proponent (last 5 years)

Gerbino, V., Kaunga, E., Ye, J., Canzio, D., O'Keeffe, S., Rudnick, N.D., Guarnieri, P., Lutz, C.M., and Maniatis, T. (2020). The Loss of TBK1 Kinase Activity in Motor Neurons or in All Cell Types Differentially Impacts ALS Disease Progression in SOD1 Mice. Neuron 106, 789- 805.e5.

Klionsky, D.J., Abdel-Aziz, A.K., Abdelfatah, S., Abdellatif, M., Abdoli, A., Abel, S., Abeliovich, H., Abildgaard, M.H., Abudu, Y.P., Acevedo-Arozena, A., et al. (2021). Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition)1. Autophagy 17.

Ye, J., Cheung, J., Gerbino, V., Ahlsén, G., Zimanyi, C., Hirsh, D., and Maniatis, T. (2019). Effects of ALS-associated TANK binding kinase 1 mutations on protein–protein interactions and kinase activity. Proc. Natl. Acad. Sci. 116, 24517–24526.

Rudnick, N.D., Griffey, C.J., Guarnieri, P., Gerbino, V., Wang, X., Piersaint, J.A., Tapia, J.C., Rich, M.M., and Maniatis, T. (2017). Distinct roles for motor neuron autophagy early and late in the SOD1G93A mouse model of ALS. Proc. Natl. Acad. Sci. U. S. A. 114, E8294–E8303.

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2. Evans CS, Holzbaur ELF (2018) Autophagy and mitophagy in ALS. Neurobiol. Dis.

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4. Gerbino V, Kaunga E, Ye J, Canzio D, O'Keeffe S, Rudnick ND, Guarnieri P, Lutz CM, Maniatis T (2020) The Loss of TBK1 Kinase Activity in Motor Neurons or in All Cell Types Differentially Impacts ALS Disease Progression in SOD1 Mice. Neuron 106:789-805.e5. doi: 10.1016/j.neuron.2020.03.005

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