

Al collegio docenti del Dottorato in Medicina Molecolare

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“Disentangling Neuronal ceroid lipofuscinosis through a zebrafish modelling”

- Introduzione

The neuronal ceroid lipofuscinoses (NCL) are a group of rare genetic neurodegenerative disorders, predominantly occurring during childhood and early adult life, multiple studies identified different forms of NCL associated with mutations in 13 genes (*cln1-cln8*, *cln10-cln14*), characterized by accumulation of autofluorescent storage material in lysosomes. The defects in neuronal lysosomal function also led to dysregulation of autophagy. The aim of my PhD project is to develop and characterized *cln1/3/5*-deficient knock-out zebrafish lines to unravel new pathomechanisms behind this disease and to test molecules through high-throughput drug screening.

- Metodiche utilizzate

Zebrafish lines were generated using CRISPR-Cas9 technology, sgRNA were designed specifically for *cln* genes. CRISPR-Cas9 and sgRNA were injected in zebrafish embryos at 1-cell stage. mRNA expression of *cln* genes has been evaluated using Real-Time qPCR. *in-vivo* imaging analysis in zebrafish embryos and larvae was used to verify the presence of apoptotic cells (Acridine orange), impaired lysosomal pH (LysoTracker) and accumulation of lipid droplets (Bodipy). Locomotion behaviour was analysed using Danio Vision system, to test frequency of tail flicking, velocity and distance moved.

- Risultati ottenuti

Valid sgRNAs with high efficiency have been identified for *cln5*, *cln3* and *cln1*. *cln5* and *cln3* expression has been found to be significantly reduced in mutated embryos compared to wild-type siblings, indicating a decrease in the mRNA production. Locomotion tests on mutant larvae show an hyperexcitability and a significant decrease in velocity and distance travelled against controls. Moreover, *in-vivo* imaging highlights an increased in the number of apoptotic cells especially in the brain region, an impairment in lysosomal pH and an accumulation of lipid droplets with a significant increase of fluorescence intensity.

Abstracts e partecipazione a congressi e corsi: autori, titolo della presentazione, nome e date del congresso

- Bernardi S., Ogi A., Mero S., Gemignani F., Santorelli F. M., Marchese M. Disentangling Batten disease through a zebrafish modelling: characterization of a new cln3-deficient zebrafish line. 18th NCL2023 Congress- Hamburg 25-30 September 23. Poster presentation.
- Bernardi S., Ogi A., Licitra R., Mero S., Galatolo D., Naef V., Gemignani F., Ratto G. M., Nardi G., Rapposelli S., Zang J., Neuhaus S., Santorelli F. M., Marchese M. Insight CLN5: Approaching therapies in the neuronal ceroid. XXI Scientific Convention Telethon, Riva Del Garda 13-15 March 23. Poster presentation.
- Pisa Zebrafish Day 3.0, Pisa 15 September 23.
- Corso di formazione in materia di protezione degli animali utilizzati a fini scientifici. Pisa, June-July 23.
- "I finanziamenti europei nel settore digitale". 11/05/23.
"Europrogettazione: programmi, progetti e rendicontazione". 18/05/23.
"Science and Society: continuity and change". 29/05/23. Università di Siena.
"PhD and then? Overview on career paths inside and outside the academia". 12/06/23.
"Materiale promozione THRUST corso incentivazione imprenditorialità Ecosistema THE". 20-30/06/23.
"Last update in Sustainability reporting regulation". 07/09/23.
"Ricerche bibliografiche, bibliometria e open access & science". 14/09/23. Università di Siena.
"The role of standardization in innovation development". 20/09/23. Università di Siena.

Pubblicazioni scientifiche autori, titolo della pubblicazione, nome e numero della rivista, anno di pubblicazione

- De Paolo, R., Sarti, S., Bernardi, S., Cucco, F., Tavosanis, A., Pitto, L., & Polisenio, L. (2023). Differential impact of BRAFV600E isoforms on tumorigenesis in a zebrafish model of melanoma. Cell & bioscience, 13(1), 121. <https://doi.org/10.1186/s13578-023-01064-w>
- Bernardi, S., Gemignani, F., & Marchese, M. (2023). The involvement of Purkinje cells in progressive myoclonic epilepsy: Focus on neuronal ceroid lipofuscinosis. Neurobiology of disease, 185, 106258. <https://doi.org/10.1016/j.nbd.2023.106258>