PROGETTO

Supervisore::	Alessandro Sessa
Titolo/*Title*:	**Exploring the epigenetic rewiring associated to *RLF* mutations as a driver of intellectual disability**
Curriculum:	Neuroscienze e Neurologia Sperimentale
Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento:	https://research.hsr.it/en/divisions/neuroscience/neuroepigenetics.html

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

De novo heterozygous mutations in the *RLF* gene have been recently found in a cohort of patients with an array of symptoms including intellectual disability (ID), recognizable facial dysmorphism, motor delay, behavioral alterations, and occasional congenital malformations. RLF protein has been involved with the regulation of critical elements of the epigenetic code which have been found altered in diseases phenotypically close to the condition of RLF patients. However, the function of RLF protein, the impact of its genetic variants, as well as the downstream molecular mechanisms are unknown in the context of the central nervous system, therefore the molecular diagnosis allows only limited therapeutic options.

Rationale and hypothesis

Re-arranged L-Myc Fusion (RLF) is a zinc finger DNA binding protein found involved in controlling the DNA methylation, and possibly also H3K4 methylation, especially at distal regulatory regions[1,2]. However, the protein is generally poorly characterized, especially in neural cells, leaving the disease without proposed molecular mechanisms. We hypothesize a functional link between DNA and histone methylation at enhancers, possibly through RLF function, underpinning the chromatin landscape of neural cells. Thus, pathological *RLF* mutations would

drive to an epigenetic rewiring either during brain development or function or both, ultimately leading to neurological defects as already seen in other chromatinopathies like Kabuki syndrome[3-5].

Objectives and specific aims

To understand the disease mechanisms, we have already generated human induced Pluripotent Stem Cells (iPSCs)[6,7] isogenic lines carrying two specific mutations found in patients as well as complete deletion of RLF, using CRISPR. Here, we aim to identify and genome-wide map at the single-cell level the epigenetic changes due to RLF variants and interpret their functional consequences in relevant experimental model using cerebral organoids[8-11].

Aim1: To describe the phenotypic features of RLF mutations in brain organoids.

Aim2: To define the functional role of RLF in coordinating chromatin regulation.

Expected outcomes

This project, if successful, will allow to gain information on the consequences of RLF loss-of-function (LoF), or malfunctioning, regarding correct cell type generation, their spatial organization as well as their basic properties. Indeed, these features may represent the ground on which the clinical relevant phenotypes can develop.

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

The student will acquire abilities in molecular cloning, IPSC culturing and differentiation, organoid generation and analysis, cytochemical analyses, Western blotting. The project will also foresee many genomic approaches: the student will be able to proficiently generate libraries ready to be sequenced, while bioinformatics analysis will be performed by a skilled bioinformatician in the lab.

The student will apply genomic techniques also at single cell levels to gain molecular understanding of the macro-phenotypes and ultimately the clinical signs of the patients.

Bibliografia (max. 15)

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11. Kanton S, Boyle MJ, He Z et al. Organoid single-cell genomic atlas uncovers human-specific features of brain development. Nature 2019; 57: 418–422. doi: 10.1038/s41586-019-1654-9.