PROGETTO

Supervisore:: _Gaia Colasante_____

Titolo/*Title*: Investigating the role of Scn1a in non-neuronal cell types in the brain

Curriculum: Neuroscienze e Neurologia Sperimentale

Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento: https://www.unisr.it/en/offerta-formativa/medicina-chirurgia/post-lauream/dottorato-medicina-molecolare/director-of-studies/colasante-gaia

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

Dravet syndrome (DS) is a severe developmental and epileptic enecephalopathy arising in the first year of life of otherwise normal babies, characterized by refractory seizures and behavioral alterations, including autistic traict, intellectual disability and cognitive impairment. It is caused by haploinsufficiency of *SCN1A* gene, that encodes for the alpha subunit of the voltage-gated sodium channel Nav1.1[1,2]. Nav1.1 is mainly localized in the axonal initial segment (AIS) and internodes of GABAergic inhibitory interneurons and it plays a focal role in both genesis and propagation of action potentials (APs) in neurons[3–6]. Its reduced expression in DS underlines low excitability of those neurons and ultimately an unbalance in the excitatory/inhibitory ratio. The final output is a strong seizure susceptibility and behavioral alterations that are well recapitulated in DS murine models. Current pharmacological treatments for DS are ineffective in completely control convulsive attacks or ameliorating neurological symptoms, therefore precision medicine approaches based on removing Scn1a gene haploinsufficiency are being conceived for DS[7-12] and the recent development of PHP.eB AAVs offers the unprecedented opportunity to transduce large areas of the brain by systemic delivery. We have recently generated a new reversible mouse model of DS (Scn1a$^{Stop/+}$)[8], that helped to prove that both seizures and behavioral alterations can be rescued upon re-expression of physiological levels of Scn1a after symptom onset in mice at postnatal day 30 (P30). While some of the gene therapies in development have been conceived to target only GABAergic interneurons[12], the expression of Scn1a has been detected also in principal neurons of cortex and hippocampus and in non-neuronal cell types of the brain that could sense haploinsuffciency of the gene.

Rationale and hypothesis

In this context, we hypothesize that the activity of non-neuronal cell types of the brain may be affected by Scn1a haploinsuffciency and may contribute to the Dravet phenotype.

Objectives and specific aims

The aim of the present project is to unravel the role of non-neuronal cell types in the pathogenesis of Dravet syndrome. To this aim, the PhD student will exploit mouse models conditionally expressing Scn1a to achieve selective gene inactivation or gene activation in specific non-neuronal brain cell types, in defined stages of the pathology (onset and chronic stage). Phenotypic and molecular analysis of those models will be performed by video EEG analysis, patch-clamp, immunofluorescence and single cell and spatial transcriptomic.

Expected outcomes

The successful accomplishment of the project will provide insights into the role of Scn1a in non-neuronal brain cell types that express the gene. Specifically, we expect to identify the contributions of glial cells of different brain areas to the Dravet phenotype in mouse models of the disease.

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

The PhD candidate will learn to manipulate murine animal models and to make genetic crossings. Moreover, he/she will learn to perform surgery for implant of EEG-transmitters and to record and analyze EEG activity. He/she will be trained to learn patch-clamp on brain slices. Transcriptomic analysis will be performed in collaboration, but the PhD candidate will learn about the experimental design and the data analysis in those experiments.

Bibliografia (max. 15)

1. Brunklaus A, Ellis R, Reavey E, Semsarian C, Zuberi SM. Genotype phenotype associations across the voltage-gated sodium channel family. J Med Genet. 2014;51(10):650-658. doi:10.1136/jmedgenet-2014-102608

2. Catterall WA. Dravet syndrome: a sodium channel interneuronopathy. Curr Opin Physiol. 2018;2:42-50. doi:10.1016/j.cophys.2017.12.007

3. Yu FH, Mantegazza M, Westenbroek RE, et al. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. Nat Neurosci. 2006;9(9):1142-1149. doi:10.1038/nn1754

4. Ogiwara I, Miyamoto H, Morita N, et al. Nav1.1 Localizes to Axons of Parvalbumin-Positive Inhibitory Interneurons: A Circuit Basis for Epileptic Seizures in Mice Carrying an Scn1a Gene Mutation. J Neurosci. 2007;27(22):5903-5914. doi:10.1523/JNEUROSCI.5270-06.2007

5. Kaneko K, Currin CB, Goff KM, Somarowthu A, Vogels TP, Goldberg EM. DEVELOPMENTALLY-REGULATED IMPAIRMENT OF PARVALBUMIN INTERNEURON SYNAPTIC TRANSMISSION IN AN EXPERIMENTAL MODEL OF DRAVET SYNDROME A PREPRINT. Published online 2021. doi:10.1101/2021.07.28.454042

6. Duflocq A, Le Bras B, Bullier E, Couraud F, Davenne M. Nav1.1 is predominantly expressed in nodes of Ranvier and axon initial segments. Mol Cell Neurosci. 2008;39(2):180-192. doi:10.1016/j.mcn.2008.06.008

7. Hsiao J, Yuan TY, Tsai MS, et al. Upregulation of Haploinsufficient Gene Expression in the Brain by Targeting a Long Non-coding RNA Improves Seizure Phenotype in a Model of Dravet Syndrome. EBioMedicine. 2016;9:257-277. doi:10.1016/j.ebiom.2016.05.011

8. Colasante G, Lignani G, Brusco S, et al. dCas9-Based Scn1a Gene Activation Restores Inhibitory Interneuron Excitability and Attenuates Seizures in Dravet Syndrome Mice. Mol Ther. 2019;28(1). doi:10.1016/j.ymthe.2019.08.018

9. Yamagata T, Raveau M, Kobayashi K, et al. CRISPR/dCas9-based Scn1a gene activation in inhibitory neurons ameliorates epileptic and behavioral phenotypes of Dravet syndrome model mice. Neurobiol Dis. 2020;141. doi:10.1016/j.nbd.2020.104954

10. Lim KH, Han Z, Jeon HY, et al. Antisense oligonucleotide modulation of non-productive alternative splicing upregulates gene expression. Nat Commun. 2020;11(1). doi:10.1038/s41467-020-17093-9

11. Han Z, Chen C, Christiansen A, et al. Antisense oligonucleotides increase Scn1a expression and reduce seizures and SUDEP incidence in a mouse model of Dravet syndrome. Sci Transl Med. 2020;12(558). doi:10.1126/SCITRANSLMED.AAZ6100

12. Tanhenaus A., et al. Cell-Selective Adeno-Associated Virus-Mediated SCN1A Gene Regulation Therapy Rescues Mortality and Seizure Phenotypes in a Dravet Syndrome Mouse Model and Is Well Tolerated in Nonhuman Primates. Hum Gene Ther. June 2022; 33(11-12): 579–597.