PROGETTO

Supervisore:: _Gaia Colasante_____

Titolo/*Title*: Investigating and enhancing the trafficking of Nav1.1 channel to set a gene therapy for Dravet Syndrome

Curriculum: Gene and Cell Therapy

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) of GABAergic inhibitory interneurons, where it plays a focal role in the genesis of the action potentials[3,4] (APs), and at nodes of Ranvier where it favours their propagation[5,6]. Gene therapy strategies aiming to rescue the haploinsufficiency of Scn1a gene by boosting the expression of the wild-type allele at both transcriptional and post-transcriptional of the gene are being developed[7–11]. However, another crucial step of Nav1.1 gene expression is its trafficking to the plsma particularly in light of the evidence that about only 30% of the channel is in the plasma membrane, while most of it is retained in intracellular membranes[12].

Rationale and hypothesis

In this context, we hypothesize that enhancing Nav1.1 trafficking to the plasma membrane might be a suitable strategy to rescue its haploinsuffciency and for the treatment of Dravet Syndrome.

Objectives and specific aims

The PhD project will develop through the following aims:

1) developing a reporter cell line to detect Nav1.1 channel localized in the plasma membrane

2) Identifying key regulators modulating Nav1.1 trafficking by gain and loss of function approaches

3) Enhancing Nav1.1 trafficking by installing specific mutations in selected residues of the channel.

4) Assessing the effect of Nav1.1 enhanced trafficking on Dravet phenotype

Expected outcomes

The successful accomplishment of the project will first generate a useful reporter of Nav1.1 localization in the plasma membrane and determine key regulators of its trafficking to specific membrane compartments. Finally, we expect to identify specific residues of Nav1.1 protein that could be mutagenized by a Base Editing approach to prevent its intracellular retainance and boost its localization to the plasma membrane, without affecting the channel properties and functionality. The final test in a cellular models of Dravet syndrome will define the feasibility to pursue this approach for a gene therapy strategy for this disease.

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

The PhD candidate will develop *in vitro* models to study Nav1.1 trafficking and distribution. He/she will be trained to performe cutting edge molecular biology techniques, including CRISPR/Cas9 screening and Base Editing strategies. In addition, he/she will learn to generate transgenic animal models and manipulate mice. He/she will learn to derive primary neurons, generate viral vectors to transduce them and exploit microfluidic devices and *in vivo* imaging.

Bibliografia (max. 15)

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12. Schmidt JW and Catterall WA. Byosinthesis and Processing of the alpha-Subunit of the Voltage-Sensitive sodium channel in rat brain neurons. Cell. 1986 Aug 1; 46(3): 437-44.