PROGETTO

Supervisore: D'Adamo Patrizia

Titolo/*Title*: RAB39B gene therapy and AMPAR modulation in a pre-clinical mouse model for Intellectual Disability

Curriculum: NEN

Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento: https://research.hsr.it/en/divisions/neuroscience/rett-syndrome-and-neurodevelopmental-disorders/patrizia-d-adamo.html

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

The most prominent neuronal phenotype in Intellectual Disability (ID), as in others neurodevelopmental disorders, is the alteration in dendritic spine development suggesting that abnormalities in dendritic spines formation, pruning and maintenance is a common hallmark for disorders that include deficits in cognition and information processing. *RAB39B* mutations cause ID, Autism Spectrum Disorders, macrocephaly and seizures[1] or early-Parkinson's Disease[2]. RAB39B, in tandem with PICK1, drives the GluA2/GluA3 AMPAR trafficking throughout the secretory pathway to achieve AMPAR subunits insertion at the neuronal post-synaptic terminal[3]. RAB39B deletion leads to an increased surface expression of GluA2-lacking Ca^{2+}-permeable (CP-)AMPAR that results in immature spine asset caused by an unsuccessful neuronal dendritic spine refinement around 30 days of age. Spine defects are associated with cognitive impairment as for *Rab39b* KO adult mice showing hyperactivity, increased curiosity, and impaired associative and working memory[4]. In rodents, the molecular mechanism underlying spines development and maturation is not totally defined thus, the importance to fully dissect the molecular networks controlling these events.

Rationale and hypothesis

We hypothesize that the absence of subunit switching from CP- to GluA2-containing Ca^{2+}-impermeable AMPAR, absent during development in *Rab39b* KO mice, results in a permanent CP-AMPAR asset determining the failure of dendritic spine pruning. The consequent increase in intracellular Ca^{2+} influx results in a more excitable synaptic network, in immature spine asset with hyper-dynamic behaviour and in cognitive alterations. Thus, the modulation of the intracellular protein cascade affected by the absence of RAB39B and then GluA2-AMPAR switching will revert spine-pruning defect and cognitive performance.

Objectives and specific aims

To achieve our aim, we will a) restore RAB39B activity by a gene therapy approach (AAVPHP.eB-CAG-humanHA-*Rab39b* injection) and b) balancing the Ca^{2+} entry by NASPM administration, followed by biochemical studies, high-resolution imaging, and behavioural studies with the final aim to discover/elucidate the correlation between deregulation of signalling pathway and synaptic dysfunction and pave the path to development of potential therapies.

Expected outcomes

The proposed experiments will be feasible in 3 years. This is a hypothesis-driven project, based on solid preliminary results, that will specifically result in 1) assessment of intracellular neuronal localization and dynamic trafficking of proteins involved in spine maturation and stabilization during development in longitudinal studies to define new molecular markers and 2) specific intervention with AAVPHP.eB-humanHA-*Rab39b* and NASPM efficiently acting on AMPAR, will likely generate positive results.

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

- primary hippocampal cell culture from E18 mouse embryos
- histological and immunofluorescence analysis
- Biochemistry, sample fractionation and westen blot
- behavioural testing

Bibliografia (max. 15)

1 Giannandrea, M., Bianchi, V., Mignogna, M.L., Sirri, A., Carrabino, S., D'Elia, E., Vecellio, M., Russo, S., Cogliati, F., Larizza, L. *et al.* (2010) Mutations in the small GTPase gene RAB39B are responsible for X-linked mental retardation associated with autism, epilepsy, and macrocephaly. *Am J Hum Genet*, 86, 185-195. DOI: 10.1016/j.ajhg.2010.01.011

2 Ciammola, A., Carrera, P., Di Fonzo, A., Sassone, J., Villa, R., Poletti, B., Ferrari, M., Girotti, F., Monfrini, E., Buongarzone, G. *et al.* (2017) X-linked Parkinsonism with Intellectual Disability caused by novel mutations and somatic mosaicism in RAB39B gene. *Parkinsonism Relat Disord*, 44, 142-146. DOI: 10.1016/j.parkreldis.2017.08.021

3 Mignogna, M.L., Giannandrea, M., Gurgone, A., Fanelli, F., Raimondi, F., Mapelli, L., Bassani, S., Fang, H., Van Anken, E., Alessio, M. *et al.* (2015) The intellectual disability protein RAB39B selectively regulates GluA2 trafficking to determine synaptic AMPAR composition. *Nat Commun*, 6, 6504. DOI: 10.1038/ncomms7504

4 Mignogna, M.L., Musardo, S., Ranieri, G., Gelmini, S., Espinosa, P., Marra, P., Belloli, S., Murtaj, V., Moresco, R.M., Bellone, C. *et al.* (2021) RAB39B-mediated trafficking of the GluA2-AMPAR subunit controls dendritic spine maturation and intellectual disability-related behaviour. *Mol Psychiatry*, 26, 6531-6549. DOI: 10.1038/s41380-021-01155-5