PROGETTO

Supervisore: Rossella Galli

Titolo/*Title*: Deconstructing the involvement of abnormally protracted corticogenesis in the etiology of cortical defects in a postnatal mouse model of Tuberous Sclerosis Complex

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/neural-stem-cell-biology.html**

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

Tuberous sclerosis complex (TSC) is an autosomal disorder caused by mutations in TSC1 or TSC2 and characterized by the presence of benign tumors in the brain, known as subependymal nodules (SENs) and subependymal giant cell astrocytomas (SEGAs). The most debilitating aspects of TSC are the neurological conditions, which include early-onset drug-resistant epilepsy (Parenti et al., 2020). The TSC pathognomonic lesions responsible for epilepsy are the cortical tubers (CTs), i.e., macroscopic regions of disorganized cortical layering that act as epileptic foci (Blair et al., 2018).

To improve mouse modeling of TSC, a few research groups, including ours, set out to restrict mTOR hyperactivation *in vivo* to postnatal subventricular zone (SVZ) progenitors (Zhou et al., 2011) (Feliciano et al., 2012) (Zordan et al., 2018). We recently interbred $Tsc1^{c/c}$ $Pten^{c/c}$ mice with $NestinCre^{ERT2}$ mice and hyperactivated both mTORC1 and Akt pathways in subventricular zone (SVZ) progenitors at postnatal day (P)10 and P15-17. By this strategy, we promoted the formation of focal SENs and full-blown SEGAs that closely resembled the lesions in TSC patients (Zordan et al., 2018). As expected, since mouse corticogenesis is known to end before birth, no cortical defects were reported in these postnatally-deleted mouse models.

Rationale and hypothesis

Given these premises, it came as a surprise the observation that, when deleting both Tsc1 and Pten at early perinatal stages in the same mouse model, mutant mice developed epileptic seizures that were associated with defects in cortical layering, thus implying that perinatal hyperactivation of mTORC1 and Akt in SVZ progenitors may contribute, either cell autonomously or non-cell autonomously, to the observed cortical disturbances.

Objectives and specific aims

The overarching goal of the project is to dissect the mechanisms underlying the SVZ-dependent neurogenic processes at the basis of the cortical defects, making available a novel and convenient postnatal in vivo asset, capable to model TSC-associated CT-like abnormalities.

To this end, the experimental strategy is subdivided into two main aims.

The first aim will focus on a thorough understanding of the cellular processes involved in the defective corticogenesis by means of an already available NestinCre^{ERT2}::Tsc1$^{c/c}$Pten$^{c/c}$R26-Confetti$^{c/c}$ mouse model in which the fate, lineage affiliation, and differentiation status of SVZ progenitors and of their progeny will be clonally traced by distinct reporter genes.

The second aim will try to disentangle the cortical cellular complexity and to decipher the gene regulatory networks underlying the cortical defects in mutant Confetti mice by single-nuclei transcriptomics.

Expected outcomes

Altogether, these experimental approaches will provide an exhaustive and timely comprehension of the cellular mechanisms and molecular mediators involved in CT etiopathogenesis. This knowledge could ultimately lead to the identification of novel druggable targets and to the development of disease-modifying treatments beyond mTOR inhibitors.

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

The student will have the opportunity to become acquainted with basic molecular and cellular biology techniques (*e.g.*, qPCR, WB, IHC, RNAi), bioinformatics analysis and imaging (*e.g.,* confocal microscopy).

Bibliografia (max. 15)

Blair, J. D., Hockemeyer, D., and Bateup, H. S. (2018). Genetically engineered human cortical spheroid models of tuberous sclerosis. Nat Med 24, 1568-1578.

Feliciano DM, Quon JL, Su T, Taylor MM, Bordey A. Postnatal neurogenesis generates heterotopias, olfactory micronodules and cortical infiltration following single-cell Tsc1 deletion. Hum Mol Genet 2012;21:799-810.

Parenti, I., Rabaneda, L. G., Schoen, H., and Novarino, G. (2020). Neurodevelopmental Disorders: From Genetics to Functional Pathways. Trends Neurosci 43, 608-621.

Zhou, J., Shrikhande, G., Xu, J., McKay, R. M., Burns, D. K., Johnson, J. E., and Parada, L. F. (2011). Tsc1 mutant neural stem/progenitor cells exhibit migration deficits and give rise to subependymal lesions in the lateral ventricle. Genes Dev 25, 1595-1600.

Zordan P, Cominelli M, Cascino F, Tratta E, Poliani PL, Galli R. Tuberous sclerosis complex-associated CNS abnormalities depend on hyperactivation of mTORC1 and Akt. J Clin Invest 2018;128:1688-706.