Proposal for the 2020 Imagine International PhD program

Laboratory: Translational Resaerch in Neurological Diseases, Institut Imagine

Head of laboratory: Edor KABASHI

Field of research: Neuroscience

Number of students accepted: 0

Supervision: Rima NABBOUT and Thomas BLAUWBLOMME

Project: Molecular and physiological targets for focal cortical dysplasia (FCD)

The most frequent histological diagnosis in pediatric epilepsy surgical resections is Focal Cortical Dysplasia (FCD), a developmental disorder defined by abnormal cortical architecture (type I), with the presence of abnormal cells in type II (cytomegalic neurons, balloon cells). Even though surgery yields excellent results in a majority of cases, deciphering the pathophysiology remains a major issue as 30-40% of the patients are not properly treated by surgery and remain drug resistant. Genetically, FCD was associated with both germline and somatic mutations in genes encoding for various components of the PI3K/AKT3/mTOR pathway leading to aberrant mTOR kinase activation. Recent work from Dr. Blawblomme has evidenced the contribution of GABAergic transmission in triggering seizures through paradoxical depolarization in FCD (Blawblomme et al., Ann Neurol, 2019). Using electrophysiological recordings in post-surgical cortical tissue from FCD patients, the team was able to precisely map the epileptogenic region and to identify a chloride misregulation pathway through the differential expression of the potassium-chloride co-transporter 2 (KCC2) and sodium-potassium-chloride co-transporter (NKCC1). This leads to the accumulation of chloride inside the cells and the resulting shift from an inhibitory (hyperpolarizing) to an excitatory (depolarizing) effect of GABA receptor activation on the cell membrane.

This translational PhD project aims to further define the cytological abnormalities in FCD and to explore the link between genetic mutations and alterations of cellular and physiological mechanisms that ultimately result in epileptic activity. Multi electrode array electrophysiological recordings under pharmacological manipulation will be used to map precisely the epileptic and non-epileptic areas in post-surgical cortical tissue slices. Further, immunolocalization of key components and molecular analysis of signaling pathways will be correlated to the epileptogenic zones. The microarchitecture of the dysplasic areas will be investigated using conventional histology and 3D imaging with clearing techniques. To pave the way for new antiepileptic drugs, the key pathways that are deregulated will be targeted in the patient's tissue *in vitro*.

AIM 1. Mapping of the epileptic activity of the FCD tissue in vitro. Thick cortical slices from resected patient tissue will be recorded on a Micro Electrode Array in ACSF. Inter-electrode intervals of 1500 µm / 1000 µm will allow to record local field potentials in a large cortical surface (>2cm). The ictal activities will be mapped to the cortical slices via an integrated camera using the MEA Monitor software (Multichannel Systems, Germany) to identify the location of the electrodes relative to the pial surface of the slices. In a subset of slices, the epileptogenic areas will be microdissected for further molecular

biology analysis. All other slices will be fixed for immunohistochemistry and 3D reconstruction.

AIM 2. Dissecting chloride regulation pathways. Antibodies against pS6, KCC2 and NKCC1 will be used in immunolabelling and Western Blotting to define the regions of mTOR hyperactivation. Confocal microscopy will be performed at the Cell Imaging platform of Imagine on the immunolabelled slices that were previously recorded on MEA. Due to the thickness of the slices (300nm), tissue clearing techniques will be explored in collaboration with the Imaging platform. The levels and phosphorylation status of WNK-OSR-SPAK kinases, responsible for the correct expression of chloride transporters, and of members of the PKC family will be assessed. We hypothesize that in epileptic regions we will observe increased mTOR activation correlating with higher phosphorylation level of OSR-SPAK along increased expression of NKCC1.

AIM 3. Effects of pharmacological modulation on neural activity. To address the regulatory cascade upstream of the chloride co-transporter activity and localization, we will use pharmacological modulation of mTOR signaling. mTOR inhibitors (Everolimus (1µM), Metformin (200µM)) and specific antagonists of the WNK pathways (STOCK1S-50699 (10µM)) will be applied to the bath perfusion for prolonged periods (>2hrs) to determine its effect on the epileptic-like activity of the tissue. Post-treatment immunohistology and proteomic studies will allow to determine: (i). the effective inhibition of mTOR by monitoring the state of S6 phosphorylation; (ii). the relative expression and localization of KCC2 and NKCC1, as well as the phosphorylation of KCC2 and (iii). the activation status of the mTOR effector pathway WNK-SPAK/OSR1 and PKC. Further, this correlative physiology-histology-pharmacology approach will allow us to rapidly test candidate therapeutic compounds arising from the identified dysregulated pathways of AIM 2.

This project is part of a research program in close collaboration with the National Center for Rare Epilepsies headed by Pr. Nabbout and the surgery unit headed by Dr. Blauwblomme at Necker Hospital. The electrophysiology and imaging platforms at Imagine will enable to perform this PhD project within the timeframe of 3-4 years.

Lab members

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Major Publications

Blauwblomme T, et al. *Ann Neurol.* 2019 Feb;85(2):204-217
Blauwblomme, Dzhala, Staley. *Ann Clin Transl Neurol.* 2018 Jul 5;5(9):1048-1061.
Dossi, **Blauwblomme** et al. *Sci Transl Med.* 2018 May 30;10(443).
Devinsky, **Nabbout**, *et al. N Engl J Med. 2017 376:2011-2020.*
Kabashi. *Lancet Neurol. 2017 May;16(5):348.*
Sellier, …, **Kabashi***, et al.***.** *EMBO J. 2016 12:1406-8.*
French, …, **Nabbout***, et al. Lancet. 2016;388:2153-2163.*