Master de Sciences et Technologies
Mention Biologie Intégrative et Physiologie
Parcours : Neurosciences
Responsable : Professeur Régis Lambert

Internship Proposal
Academic Year 2019-2020

1. Host team :

Research Unit (e.g. Department or Institute): Institut du Fer à Moulin, Inserm UMR-S 1270
Research Unit Director: Jean-Antoine GIRAULT
Research Team Director: Fiona FRANCIS / Laurence GOUTEBROZE
Team name: Cortical development and pathology

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2. Internship project title:

Neural progenitor cell dynamics during normal and abnormal mouse brain development

3. Internship Description :

Abnormalities of cortical development are important causes of epilepsy and intellectual disability. These can arise through perturbed progenitor function, neuronal migration and/or connectivity. Mutant genes identified in cortical malformations provide new entry points for exploring key cellular and subcellular mechanisms of brain development. We use mouse models, combined with state-of-the-art imaging, to decipher the roles of proteins in progenitor cells and migrating neurons *in vitro* and *in vivo*.

This new project is focused on the EML1 microtubule (MT) cytoskeletal protein. *Eml*1/ *EML*1 mutations are associated with abnormal neuronal position (severe heterotopia) in rodent and human brains. We showed that this phenotype is linked to progenitor cell abnormalities (Kielar et al, Nat Neurosci, 2014). During mouse embryogenesis, radial glial progenitor cell (RG) soma are normally present in the ventricular zone (VZ) of the developing cortex, however a proportion of Eml1 mutant cells are ectopic in the mutant brain. These cells are highly polarized and also serve as guides for migrating neurons. Abnormal mutant RG position affects subsequent neuronal migration and contributes to heterotopia formation. But the role of Eml1 in RGs is completely unknown and this project addresses the perturbed progenitor mechanisms in mouse mutants. **Few groups focus on the maintenance or detachment of progenitors in the VZ and the M2 student will explore these mechanisms in detail.** We have excellent tools and expertise (constitutive and conditional mouse models, the in utero electroporation technique, constructs coding for different fluorescent proteins, confocal and spinning disk microscopy) to explore early cortical developmental defects leading to heterotopia. **The aims of this project will be to: 1) question the role of Eml1 in RGs, since it is expected to normally prevent RG detachment from the VZ; 2) assess how heterotopic cells cluster in subcortical regions by real-time imaging.**

For **aim 1**, we have already shown that Eml1 is a conserved MT-binding protein which in dividing RGs associates with the mitotic spindle (Bizzotto et al, Sci Rep, 2017). However, it is also present during interphase and there are perturbed centrosomes and primary cilia in *Eml1* mutant apical endfeet.

Abnormal endfoot detachment may occur during interphase, and videomicroscopy is already being used to confirm that this is the case. **The M2 student will focus on exploring perturbed subcellular mechanisms associated with cell detachment.** This will shed further light on the role of Eml1. Combined use of mutant mice, genetic tools, in utero electroporation and videomicroscopy will help to assess MT-dependent mechanisms in progenitors. Thus, adhesion complex turnover and membrane trafficking, as well as Golgi and centrosome behavior, need to assessed, either directly in brain slices, or in cells in culture. Primary cultures of mutant and control progenitor cells can also be used to more finely dissect perturbed mechanisms, e.g. adhesion molecule recycling, vesicle and organelle trafficking after depolymerization/ repolymerization of MTs. **Aim 2** will be performed by tracking with live-imaging fluorescently labeled ectopic progenitors in brain slices. This will reveal if neurons generated by these cells are destined for the heterotopia or overlying cortex. Data generated will reveal novel aspects of how heterotopia form in the complex 3D environment of the mouse embryonic brain.

Parallel experiments using human induced pluripotent stem cells with *EML*1 mutations, differentiated into cortical neuronal progenitors, will allow us to compare human versus mouse mechanisms (collaboration J. Ladewig, Germany). Different datasets (mouse transcriptome, Eml1 biochemical interacting partners, as well as exome-sequencing for similar severe human cortical malformations) will allow us to pinpoint Eml1-pathways in progenitors (already ongoing, Romero et al, in preparation; Uzquiano et al, submitted). Single cell RNA sequencing data will also be generated in collaboration with D. Jabaudon (Geneva Switzerland), comparing cortical progenitor cells in wild type and mutant mice. In the future in the framework of a PhD project, gain and loss-of-function experiments in the mouse will help validate important molecules regulating VZ progenitor behavior, and heterotopia formation. We will explore how perturbations of cell-cell and cell-extracellular matrix signaling contribute to these phenotypes. Our data will hence shed new light on how progenitor characteristics regulate overall brain morphogenesis from early corticogenesis.

This work is performed in close collaboration with clinical and cell biology groups (N. Bahi-Buisson, Paris; J. Ladewig, Germany, S. Cappello, Germany, D. Jabaudon, Switzerland). We also collaborate with J-B Manent (INMED, Marseille) for electrophysiology experiments in *Eml*1 mouse mutants. Our combined experiments will help elucidate critical MT-dependent processes in key progenitor cell types during brain development, as well as structural aspects of heterotopia formation which may have an impact on neuron and circuit function.