Abnormalities of cerebral cortical development are important causes of epilepsy and intellectual disability. These can arise through perturbed progenitor proliferation, neuronal migration and/or connectivity. Our group questions the neurodevelopmental functions of key microtubule (MT) cytoskeleton proteins e.g. EML1, TUBA1A, LIS1, DYNC1H1 and DCX using genetics, biochemistry and cell biology. We use mouse models and cell cultures (including human), combined with state-of-the-art imaging and analyses, to decipher the roles of these proteins in neural cells in vitro and in vivo.

This new project is focused on the Eml1 microtubule-associated protein. We previously showed that EML1/Eml1 mutations are associated with abnormal neuronal position (heterotopia) in rodent and human brains, notably and unexpectedly associated with progenitor abnormalities (Kielar et al., 2014, Nat Neurosci). Radial glia progenitors (RGs), key elements of cortical development, show abnormal positions in the Eml1 mouse mutant cortical wall, potentially due to cell detachment. The role of Eml1 in these cells is vastly unknown and this project addresses the mechanisms involved. Based on preliminary observations, the PhD student will focus on deregulated ribosomal genes and proteins found in Eml1 mutant RGs, to assess their role in causing the progenitor phenotype. This is a highly novel project, since ribosomal abnormalities in progenitor cells have not previously been associated with this type of cortical malformation and these mechanisms are little studied. Since RG detachment mechanisms are a key target of evolution, this project may shed light on critical neurodevelopmental processes influencing increased brain size, as well as pathology.

Several pieces of preliminary data have lead us to suspect that ribosomes are deregulated in Eml1 mutant situations: 1) electron microscopy experiments have shown abnormally high densities of polysomes in apical regions of mutant RGs; 2) proteomics screens for Eml1 interactors have identified ribosomal subunit proteins, of which several apparently bind less efficiently to mutant Eml1 incorporating a patient missense mutation; 3) single cell RNA sequencing data, comparing wild-type and Eml1 mutant cells, revealed a deregulation of ribosomal genes in RGs; 4) RG proliferation (dependent on protein synthesis) is altered. There is also a suggested link...
between Eml family members and ribosomal attachments to microtubules (Surprenant et al. 1993). Thus we hypothesize that Eml1 plays a role trafficking ribosomes to different regions of the RG, where they may be required for local translation. Indeed, Eml1 is known to influence both microtubules and trafficking (Bizzotto et al., 2017; Uzqian et al., 2019), lending support to this hypothesis.

The PhD student will question: 1) If Eml1 interacts with ribosomes and is likely to influence their biogenesis and / or trafficking on microtubules; 2) How Eml1 mutation perturbs ribosomes in mouse RG and human in vitro cells, related to cell phenotypes; 3) If restoring normal ribosome levels, position or activities restores RG function in the Eml1 mutant.

In a first set of experiments, biochemical approaches (immunoprecipitation) will be used with selected individual ribosomal subunit proteins of interest to confirm their interaction with Eml1. Using immunofluorescence experiments and confocal microscopy, antibodies directed against ribosomal subunits will be used in wild-type and Eml1 mutant cells (in culture and in brain tissue) to assess position and expression levels of the subunits. Particular attention will be paid to association with microtubules. Spinning disk live-imaging will be performed in wild-type versus Eml1 mutant cells (mouse and human), to follow a GFP-tagged ribosomal subunit moving along microtubules. Eml1 mutation may increase, slow or inhibit ribosomal movement.

Secondly, sucrose gradient fractionation of ribosomes will be performed from wild-type and Eml1 mutant dissociated RG (collaboration Célia Plisson-Chastang, CBI Toulouse). This will indicate if ribosome biogenesis is affected, altering ratios of large and small subunits. We will also assess translation, taking into account global, RG basal and apical levels The O-propargyl-puromycin Click-It technology (Iwasaki and Ingolia, 2020, Thermofisher) will first be applied to RGs in culture, in order to assess nascent polypeptide synthesis. Local translation in basal extremities of RGs in brain tissue has previously been demonstrated (Pilaz and Silver, 2017). We will use the same techniques of basal extremity capture, followed by transcriptome / translatome techniques (Rannals et al., 2016) from wild-type versus mutant cells. Candidate basally translated proteins will also be assessed by immunohistochemistry. Apical translation has not yet been reported, however we identified an accumulation of polysomes in apical regions, leading us to believe that this may occur. Proteins involved in apical detachment could also explain the RG phenotype in mutant mice. Using flow cytometry from dissected mouse embryonic cortical tissue we will capture RG apical endfeet in order to perform transcriptome/ translatome studies. These experiments, likely to reveal novel data, will tell us if Eml1 mutation affects locally translation, potentially linked to the phenotype.

Thirdly, using in utero electroporation in the developing mouse embryonic cortex, we will manipulate gene expression of key translated proteins, with a view to influencing RG attachment in Eml1 mutant conditions. This has already been shown in control conditions, manipulating levels of genes such as Trnp1 and Tbc1d3 (Penisson et al., 2019). Cross-comparing our protein datasets (proteomics to identify Eml1 interactors and translatome results generated in the PhD project), we will select proteins to alter their expression (knockdown or overexpression) in the mouse brain, and then verify proliferation characteristics and RG position. Thus, as well as learning more about Eml1-related pathways important in RG, we may be able to rescue the RG detachment phenotype, the primary event causing the heterotopia.

Our combined experiments will thus help elucidate critical MT-dependent ribosomal processes in a key progenitor type during the development of the brain.

Faisabilité du projet de thèse (1/2 page maximum, en anglais)

Explicit la faisabilité du projet en terme d'expertise de l'équipe d'accueil, des collaborations potentielles qui pourront être mises en place pour certains aspects du projet, de la disponibilité des appareils nécessaires au bon déroulement du projet...

The Inserm team, «Cortical development and pathology» at the UMRS-1270 is localized at the Institut du Fer à Moulin, Paris. This institute is dedicated to the development and plasticity of the nervous system, and core facilities include an animal experimentation platform with an adapted mouse surgery room, and an imaging platform with state-of-the-art microscopes, including a spinning disk microscope for dynamic studies, other confocal microscopes which will help us identify disrupted subcellular processes (e.g. including local translation and ribosome activity in apical processes). We have authorizations from the local ethical committee for our animal experimentation. This project has already been initiated in the lab by the PhD candidate (Valeria Viola) during her Master 2 internship. There are no major risks associated with this work since the mouse models are already available and pertinent data have already been generated with key phenotypic data initiating the project.

Specific people contributing to this project are F. Francis (DR1, PI), L. Goutobroze (DR2 CNRS), R. Belvindrah (MC Lecturer), D. Zaidi (PhD student), K. Chinnappa (post-doc), G. Granenc (Assistant Ingénieur, Sorbonne University) and M. Nosten-Bertrand (CR1 Researcher). Kaviya Chinnappa and Donia Zaidi will aid Valeria Viola with her PhD experiments. Julien Ferent a former post-doc and now a young principal investigator in the institute has been instrumental in supervising V. Viola and will be available for continued discussions if these are necessary. Our group already has extensive experience analyzing cortical progenitors and neuronal migration in the developing mouse embryonic cortex after in utero electroporation, including analyses of cell compartments and organelles in progenitors in brain slices, and live imaging of...
moving neurons, whilst studying organelle movement (centrosomes and microtubules, Belvindrah et al., 2017; Bizzotto et al., 2017). These approaches will be applied to this new ribosome project. We have recently acquired plasmids and antibodies required for the project. Concerning molecular mechanisms, we are well advanced in this area with several datasets (bulk and single cell transcriptome, mass spectrometry for interacting partners) already generated. We have the tools in-house for translatome experiments. We expect no problems characterizing the critical roles of Eml1 linked to ribosomes in RGs.

This project will be carried out in close collaboration with clinical and cell biology groups in the framework of an Eranet E-Rare-3 project (HETER-OMICS, coordinator F. Francis, starting date 01/06/2019) involving D. Jabaudon (Switzerland), S. Cappello (Germany), J-B Manent (Marseille, France), N. Ozlu (Turkey), M. Budisteanu & A. Arghir (Romania). These people are involved in human cell biology, neurobiology, biochemistry, bioinformatics, genetics and clinical collaborations. A visit of the PhD student to a different European lab is possible, e.g. to perform ribosome experiments in human in vitro cell cultures. A new Equipe FRM project (start 01/03/2021 - 2024) also fully supports this project. We have also initiated a collaboration with Célia Plisson-Chastang (Toulouse), a ribosome expert.

Thèses actuellement en cours dans l’équipe

Tous les encadrements doivent être indiqués (y compris les co-directions avec un autre HDR pour des doctorants d’une autre ED, et les encadrements dans le cadre de programmes doctoraux tels qu’IPV, FDV...)

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Trois publications récentes du directeur de thèse (du co-directeur ou du co-encadrant s’il y a lieu). Mettre en gras le nom du directeur de thèse.


Docteurs encadrés par le directeur de thèse ayant soutenu entre la date de dépôt de ce dossier et il y a 5 ans et publications relatives à leur sujet de thèse. Mettre en gras le nom du directeur de thèse et celui du docteur.

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Penisson, M, Hirotsune H, Francis F, Belvindrah R. Lis1 mutation prevents basal radial glia-like cell production in the mouse. https://www.biorxiv.org/content/10.1101/2020.10.10.334508v1 (Preprint and submitted)


Nom Prénom : UZQUIANO Ana
Date de soutenance : 05/2019
Durée de thèse (en mois) : 44 mois
Ecole Doctorale :
Publications :


Nom Prénom : BIZZOTTO Sara
Date de soutenance : 24 juin 2016
Durée de thèse (en mois) : 45 mois
Ecole Doctorale : CdV
Publications :
