Ecole Doctorale COMPLEXITE DU VIVANT – Fiche Projet CONCOURS

Fiche à nommer selon le format Nom_Prénom_ProjetED2021, à enregistrer en format PDF et à renvoyer à l’adresse : edcdv@sorbonne-universite.fr

Nom et prénom du directeur de thèse (et si besoin du co-directeur) : DURAND Beatrice
Le directeur de thèse et le co-directeur doivent impérativement avoir l'HDR ou équivalent
Coordonnées Tel : 0675022669 e-mail : beatrice.durand@sorbonne-universite.fr

Nom et prénom du co-encadrant (nonHdr) (s’il y a lieu) :
Coordonnées Tel :
e-mail :

Y-a-t-il un candidat déjà identifié pour le projet : OUI X NON

Nom et prénom du responsable de l’équipe : De-LI SHI

Intitulé de l’équipe : Induction and Differentiation au Cours du Développement Embryonnaire des Vertébrés

Nombre de chercheurs et enseignants-chercheurs statutaires de l’équipe titulaires d’une HDR (ou équivalent) : 2

Nom et prénom du responsable d'UMR ou de département : Sylvie Schneider-Maunoury

Intitulé et N° d’UMR ou de département : CNRS UMR7622 Laboratoire de Biologie du Développement

Titre du projet de thèse : Large scale analysis of cerebellar Granular Neuron's proliferation brake(s): Cross talks between the Notch, Sonic HedgeHog and Wnt pathways

Signature du directeur d'UMR ou de département (vaut avis favorable pour le dépôt du projet) :

Spécialité : Biologie du Développement

Résumé du projet de thèse (1 page maximum, en anglais)

During development stem/progenitor cells progressively lose their multipotence, they stop proliferating and acquire their final fate. Neurons of our highly complex nervous system show no exception to this rule, and around the time of birth most neurons are fully differentiated with one notorious exception: the Granular Neurons (GN) population that is the largest neuronal population in the brain. Indeed, the cerebellar granular neuron’s progenitor (GNPs) population is characterized by a very long period of quiescence occurring before birth, followed by a long proliferative phase – i.e. 2 weeks in mouse, 2 years in human - occurring after birth, before their final differentiation step. Due to this developmental specificity, this population is at risk when it comes to the appearance of developmental defects, including oncogenic events. What underlies this remarkable developmental feature is, at this day, an unresolved paradigm. Our project aims to shed light on the role of a highly evolutionary conserved homeodomain transcription factor BARHL2 together with its partners the T-Cell factors (TCF) in self renewal, quiescence and proliferation of the cerebellar GN stem/progenitors.

In the rodent brain, the cerebellar upper Rhombic Lip (uRhL) is a germinative area producing the GNPs. Three important signaling pathways (Notch, Sonic Hedgehog (SHH) and Wnt/TCF) interact in regulating the continuum multipotence/commitment/proliferation steps in these cells. In early GNPs, Notch signalling participates to GNPs self-renewal. At birth the RhL stem/progenitors cells become responsive to secreted SHH that promotes their survival and stimulates their proliferation (reviewed in ¹). The transcription Factors TCF, are downstream component of the Wnt canonical pathway. TCF transcriptional activity is switched on by a Wnt-driven increase of β-catenin nuclear levels ². In the Wnt ‘‘off’’ state, the TCF interact with Groucho (GRO)/Transducin-like Enhancer-of-split (TLE) proteins that can recruit histone deacetylases (HDAC), which establish regional repressive chromatin structures. Analysis of TCF activity in pluripotent versus somatic cells indicate that TCF mostly act by changing the state of chromatin in such a way that the expression of several pluripotency-related genes is switched off (Reviewed in ³). Whereas the uRhL cells exhibit positive TCF transcriptional activity, the role(s) of TCF activity and its probable interaction(s) with the Notch and the SHH pathways in the biology of GNPs are not deciphered.

The homeodomain containing transcription factor (TF) BARH-like 2 (BARHL2) is a target gene of MATH1 (ATOH1), the master gene of GNPs development ⁴. Barhl2 is expressed early on in a subpopulation of GNPs, and a recent single-cell transcriptional analysis of cerebellar development reveals a unique and high correlation of barhl2 expression with the
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emergence of the GNPs population. We recently demonstrated that BARHL2 stabilizes formation of a complex containing TCF and GRO, that further recruit HDACs. In agreement with barhl2 expression pattern we hypothesize that in the cerebellar uRhL, BARHL2 limits TCF transcriptional activation thereby locking uRhL stem/progenitor cells in a GN committed state associated with maintenance of their self-renewal properties, and changes in GNPs cell cycle properties. Our preliminary data using shRNA-mediated down regulation of Barhl2 expression in GNP primary cells together with RNA-seq analysis indicates a crucial role for BARHL2 in modulating the Notch, the SHH, and the TCF pathways and maintenance of GNPs in a self-renewal, quiescent low proliferating state.

During the course of this PhD project, we will:
1. Validate our RNA-seq observations and specifically the contribution of Barhl2 potential candidate target genes in driving self-renewal, quiescence and proliferation of cerebellar GNPs. We shall focus on Notch, SHH, and TCF target genes.
2. Perform and analyze ChIP-seq using Histone Acetylation marks (H3K27AC), together with Barhl2 and TCF as baits, thereby identifying direct or indirect target genes of Barhl2 and/or TCF and the probable contribution of HDAC activities in these transcriptional regulations.
3. Validate identified transcriptional cross-interactions between the Barhl2/TCF and the Notch and SHH pathways in GNPs.

Our observations, together with published results, are in agreement with a long-range activity of Barhl2 via its specific binding on DNA, maybe on super-enhancers as previously suggested. Understanding Barhl2 DNA binding specificity alone or together with TCF and/or HDAC should shed light on the transcriptional commitment locks at play during cerebellar development. Barhl2 deregulation is associated with emergence of a subtype of medulloblastoma, a pediatric tumor of cerebellar origin. By studying the transcriptionic and epigenetic mechanisms that control commitment of GNPs, we shall gain insight into how these processes become corrupted in cancer and use this information to develop novel ways to target tumor cells.

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...

Our team has developed in depth knowledge in theoretical concepts regarding i) developmental signalling pathways including the SHH, and Wnt/TCF pathways, ii) rodent cerebellar development which developmental steps are well characterized at the cellular and molecular levels. We have the necessary technological expertise in molecular and cellular biology, in biochemistry and in developmental embryology. We tested and produced lentiviral vectors that deliver shRNA to stably and efficiently extinguish barhl2 expression in neurons and their progenitors in a non-toxic manner. We shall similarly generate lentivirus expressing shRNA targeting TCF3 to perform TCF3 loss of function in GNPs. Using rodent GNPs primary cell culture, we demonstrated a role for BARHL2 in limiting the SHH driven GNP’s proliferative response. Using RNA-seq approaches we identified new BARHL2 target genes whose contribution to the control of GNPs quiescence, and proliferation, together with Notch, and SHH signaling are currently investigated. Flow cytometric analysis of GNP’s cell cycle parameters are ongoing in the IBPS with the help of L Petit (T. Jaffredo’ team). Our RNA-seq analysis was performed on the Curie Institute NGS platform (resp: S Baulande). Data analysis was done with the Curie Bioinformatic team (R. Montagne - resp: N Servant). Our previous work in Xenopus embryos provided proofs of concept that i) BARHL2 and TCF can activate the same set of targets genes, and that ii) HDAC participates in closing the chromatin upon recruitment of the TCF/BARHL2/HDAC complex (Sena et al, 2019). To document these observations, we are currently establishing GNP’s ChIP-seq approaches using Histone Acetylation marks or transcription factors (BARHL2, TCF712) as baits. Sequencing and bioinformatics analysis will be performed in collaboration with the Curie Institute with tutoring by the ArtBio platform (C. Antoniewski - IBPS). Investigation of potential interactions between the Notch and TCF pathways shall be performed in collaboration with the team of B. Hassan (ICM). Our project received funds by the Ligue Nationale Contre le Cancer - Comité Ile de France.

Thèses actuellement en cours dans l’équipe

emergence of the GNPs population. We recently demonstrated that BARHL2 stabilizes formation of a complex containing TCF and GRO, that further recruit HDACs. In agreement with barhl2 expression pattern we hypothesize that in the cerebellar uRhL, BARHL2 limits TCF transcriptional activation thereby locking uRhL stem/progenitor cells in a GN committed state associated with maintenance of their self-renewal properties, and changes in GNPs cell cycle properties. Our preliminary data using shRNA-mediated down regulation of Barhl2 expression in GNP primary cells together with RNA-seq analysis indicates a crucial role for BARHL2 in modulating the Notch, the SHH, and the TCF pathways and maintenance of GNPs in a self-renewal, quiescent low proliferating state.

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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...)

<table>
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<th>Nom et Prénom du doctorant</th>
<th>Directeur(s) de thèse</th>
<th>Année de 1ère inscription</th>
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<td>Bou-Rouphaël Johnny</td>
<td>DURAND Béatrice</td>
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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.

<table>
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<th>Nom Prénom : DE SENA Elena</th>
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Publications :


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<th>Nom Prénom : JURAVER-GESLIN Hugo</th>
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Publications :


3-Hugo A Juraver-Geslin¹, Jérôme J Ausseil², Marion Wasséf³ and Béatrice C Durand¹†, “Barhl2 limits growth of the diencephalic primordium via Caspase3 inhibition of β-catenin activation” 2011 Proc Natl Acad Sci U S A 108, 2288 (Feb 8, 2011).