

EQUIPE 1 :

1- Development of peptides inhibiting the isomerase activity in the treatment of macular dystrophies
(Développement de peptides inhibant l'activité isomérase dans le traitement des dystrophies maculaires)

Groupe : **Génétique et thérapie des rétinopathies pigmentaires**. Responsable : Christian Hamel.

Encadrant : Philippe Brabet.

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Projet de Recherche :

The visual cycle is an enzymatic process which allows for the regeneration of chromophore after a light stimulation of photoreceptors. The genes involved in this cycle are distributed in the photoreceptors and retinal pigment epithelium (RPE) and are mutated in various types of pigmentary retinopathies generally leading to blindness. RPE65 is the isomerase of this cycle that catalyzes the limiting step of conversion of vitamin A to chromophore. The macular dystrophies are characterized by an impairment of the RPE, which is due to the accumulation of a fluorescent pigment of lipofuscin. An innovative therapy is to limit the accumulation by slowing the visual cycle. The proof of concept of the effectiveness of such treatment was made in mice with compounds limiting the supply of vitamin A to the eyes, or inhibiting the isomerase. We have shown that the FATP1 protein could interact with RPE65 and play the role of inhibitor of isomerase. The molecular interaction between FATP1 and RPE65 is by protein domains privileged. The kinetics of inhibition of the isomerase activity by FATP1 is consistent with a mechanism of inhibition by protein interaction.

We will look more specifically to the area of interaction in FATP1 using yeast and we will attempt to identify peptides able to interact specifically. These peptides will be then tested for their ability to inhibit the production of 11-cis retinol measured in cellular models of overexpression. The accessibility of peptides to the enzyme is made possible by the use of membrane preparations for a direct measurement of isomerase activity, or by the use of CPPs (Cell Penetrating peptides) which are very promising new tools for the entry of various biologically active molecules in the RPE.

2- Identification of genes in inherited pigmentary retinopathy (identification des gènes responsables des rétinopathies pigmentaires)

Groupe : **Génétique et thérapie des rétinopathies pigmentaires**. Responsable : Christian Hamel.

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Projet de Recherche :

Non syndromic retinitis pigmentosa (RP), the most frequent form of pigmentary retinopathies, is a neurodegenerative disease with a prevalence of 20,000 to 30,000 patients in France. This condition leads to blindness in almost 100 % of cases. There is no treatment available but current research like pharmacological or gene therapy is based on the knowledge of the responsible gene in each family. Most cases are inherited as an autosomal trait, either dominant (25-30 %) or recessive (50-60 %), or follow X-linked (15 %) or complex (rarely) inheritance patterns. In large series of patients, mutations in the 50 currently known genes are found in 60 % of patients with autosomal dominant (AD) RP, and in less than 50 % in autosomal recessive (AR) RP, suggesting that several dozen of genes remain to be found.

Our laboratory is actively engaged in finding new RP genes. More than 700 RP families have been registered in the center for rare diseases of Montpellier, and DNAs have been collected for molecular genetics studies. Linkage analysis studies are ongoing through a National PHRC for 250 AD RP families and through an e-Rare program for 2,000 AR RP families. This led to screen 380 families in the EYS gene and to find 2 new loci for AR RP.

The master student will be involved in linkage mapping using genotyping with Affymetrix 250K microchips and gene screening by PCR sequencing and real time PCR. Expression of new genes will be analyzed by RT-PCR and subcellular localisation will be evaluated by *in vitro* expression in COS cells.

3- *In vitro* gene therapy for hereditary retinal dystrophies using disease-specific induced pluripotent stem cells (iPS) from patients (Thérapie génique *in vitro* dans les dystrophies héréditaires de la rétine à partir de cellules souches de patients pluripotentes induites (iPS) spécifiques de la maladie)

Groupe : **Génétique et thérapie des rétinopathies pigmentaires**. Responsable : Christian Hamel.

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Projet de Recherche:

The retina is an excellent target organ for gene therapy as it is small, relatively immunoprivileged and can be targeted by non-invasive methods. The first human gene therapy trial for a retinal disease was performed in 2008 in patients with Leber congenital amaurosis, a severe retinal dystrophy resulting from rapid loss of photoreceptors (PR). An adeno-associated virus serotype-2 vector (AAV-2) was used to introduce the missing RPE65 protein into the retinal pigment epithelium (RPE) of patients. The encouraging results provided the proof-in-principle that gene transfer can ameliorate/restore sight in visually impaired subjects and have now paved a rapid path towards clinical trials for other retinal diseases.

Prior to the use of a gene therapy vector in the clinic, pre-clinical trials are usually performed on small (e.g. mouse) and large (dog) animal models. However, in some cases, generated mouse models have proven to be asymptomatic or lethal, or the identification of a corresponding dog model has proven elusive. Therefore, human cell culture is becoming an essential complement to animal disease research. A new and exciting tool for such studies is induced pluripotent stem cells (iPS), which are capable of differentiating into any tissue. This proposal is aimed at developing disease-specific iPS lines from skin fibroblasts of patients with hereditary retinal dystrophies, which are ideal candidates for retinal gene transfer and for which an appropriate small or large animal model does not exist (choroideremia, Usher syndrome type 1 and some genetic forms of retinitis pigmentosa). Once generated these iPS lines, can then be differentiated *in vitro* into specific retinal tissues (the RPE or the PR depending on the disease phenotype). In addition to providing an insight into disease pathophysiology in a human system, the differentiated cell lines can be used for *in vitro* gene transfer studies.

The recruited student will be a key player in **i**) the generation of the iPS lines and **ii**) provoking their differentiation into RPE or PR. The long-term aim of the project is to generate viral vectors and perform *in vitro* gene transfer studies to evaluate the restoration of a normal cellular phenotype. This highly innovative project is at the forefront of stem cell technology and could have clinical repercussions in the relatively near future. Our close association with the reference centre for rare sensory disorders and the Institute for Research in Biotherapy, both at the Hôpital St-Eloi, provide an ideal setting for this project.

4- Role of OPA1 in the maintenance of mitochondrial genome integrity (implication d'OPA1 dans la maintenance de l'intégrité du génome mitochondrial)

Groupe : **Génétique et thérapie des neuropathies optiques héréditaires** Responsable : Guy Lenaers.

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Research proposal:

OPA1 is the major gene responsible for dominant optic atrophy (DOA), a blinding disease caused by the degeneration of the retinal ganglion cells. Recently, we identified a syndromic form of DOA, associating neurosensory deafness, myopathy and peripheral neuropathy, due to the presence of deletions in the mitochondrial DNA (mtDNA) and to specific mutations in OPA1 (Amati-Bonneau, Brain, 2008). This was the first report linking OPA1, encoding an inner mitochondrial dynamin, to the maintenance of mtDNA integrity.

To gain insight in this process, we developed a fundamental study to evaluate the involvement of the different OPA1 isoforms in mitochondrial genome maintenance. Our results clearly show that one OPA1 isoform is involved in the initiation of mtDNA replication, whereas another one is involved in nucleoid segregation after replication completion. Both aspects are critical considering the dramatic diseases associated to loss of mtDNA integrity (syndromic DOA and mtDNA depletion syndromes).

The master student will be involved in this study, in order to understand how mitochondrial dynamic drives mtDNA replication and integrity maintenance. The project will mainly include cell biology experiments on HeLa cells, using siRNA and plasmid transfections, immunofluorescence and mitochondrial dyes (mitotracker, JCI, picogreen), and genetic screening of patient DNA samples.

EQUIPE 3:

Canaux ioniques et mécanotransduction lors de la pousse neuritique induite après lésion nerveuse des neurones sensitifs (Ionic channels and mechanotransduction during the neurite growth following nerve injury of sensory neurons)

Groupe : **Physiopathologie du système somatosensoriel**. Responsable : Frédérique Scamps.

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Objectifs et Description : Suite à une lésion nerveuse, les neurones sensitifs modifient leur répertoire moléculaire dans le but de permettre la repousse axonale. Alors que nos données suggèrent que l'homéostasie ionique est impliquée dans ce processus, son rôle n'est pas compris. Les objectifs du projet sont 1) d'identifier les canaux ioniques sensibles aux stimuli mécaniques et en particulier les canaux chlorures ; 2) de déterminer les voies de mobilisation du calcium intracellulaire lors de la pousse régénérative ; 3) d'étudier les mécanismes de sécrétion neuronale associés au stretch mécanique.

Techniques : culture primaire de neurones sensitifs, stimulation mécanique (nanomoteur), électrophysiologie, fluorimétrie calcique et chlorure, ARN interference, électroporation, souris KO.

Following nerve injury, sensory neurons change gene pattern of expression in order to allow axonal growth. We have shown that ionic homeostasis is involved in this process, but its role is not understood. The objectives of the project are 1) Identification of ionic channels activated with mechanical stimuli with special attention to chloride channels; 2) Determination of intracellular calcium variations during regenerative growth; 3) Analysis of mechanisms of neuronal secretion associated with mechanical stretch.

EQUIPE 4 :

1- Division asymétrique et Différenciation forcée des cellules souches cancéreuses du cerveau (glioblastomes) (Asymmetric division and forced differentiation of brain cancer stem cells (glioblastoma))

Groupe : **Cellules souches neurales**. Responsable : JP Hugnot.

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Descriptif du stage : Les glioblastomes sont les tumeurs les plus agressives du cerveau. Une des avancées majeures est la mise en évidence dans ces tumeurs de cellules souches cancéreuses possédant des propriétés de résistance et de tumorigénicité plus importantes que les autres cellules. Bien qu'elles soient cancéreuses, ces cellules souches sont capables de se différencier en neurones et cellules gliales. Nos travaux visent à comprendre au niveau moléculaire 1/ comment ces cellules persistent, 2/ comment se différencient-elles ? 3/ comment forcer leur différenciation pour aboutir à un traitement ? Pour cela, 1-nous étudions en particulier la division asymétrique, un mécanisme cellulaire clé de la formation de la diversité cellulaire dans le monde vivant. 2-l'influence sur les cellules souches de la modification de l'expression de gènes de développement du système nerveux (gain et perte de fonction). Le stage de M2R portera sur l'une de ces thématiques.

Techniques : vidéomicroscopie, culture de cellules souches, biologie moléculaire, lentivirus, immunofluorescence, cytométrie, shRNA.

Description: Glioblastoma are the most aggressive brain tumor. One of a main advance is the discovery of cancer stem cells which are more resistant to chemo and radiotherapy. Although cancerous, these cells can be differentiated into glial and neuronal cells. Our work aims at understanding 1/ how stem cell persist in the tumor 2/ how they spontaneously differentiate 3/ how to force their differentiation to generate a treatment. We are studying 1/asymmetric division, a key process by which cell diversity is formed during development and 2/ influence on stem cells of key developmental genes using a gain or loss of function approach. M2R student will be involved in one of these themes.

Technics: videomicroscopy, stem cell cultures, molecular biology, lentivirurs, immunofluorescence, cytometry, shRNA.

2- Pharmacologie des gliomes (Offre de stage de recherche pour un étudiant vétérinaire)

Groupe : Motoneurone. Responsable : Norbert Bakalara. (dans l'équipe d'Alain PRIVAT)

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Durée du stage :

-stage court Juin-juillet 2009 ou à partir de la rentrée de septembre 2009

-ou stage de Master M1 ou M2

Résumé du sujet :

Le projet porte sur l'évaluation de nouvelles molécules à visée anticancéreuse *in vitro* et *in vivo*. Dans ce cadre, la caractérisation de la cytotoxicité de ces produits a été évaluée sur une lignée de type gliome (C6) et 5 produits ont été sélectionnés pour leur toxicité. Parmi ceux-ci, un produit présente la solubilité nécessaire à des expériences *in vivo* et nous souhaitons dans un premier temps évaluer la toxicité de ce produit en toxicité aiguë et répétée par voie intrapéritonéale chez des souris Nude. Les souris Nude seront par la suite utilisées comme modèle de glioblastome (gliome de grade IV) après greffe de lignée tumorale gliale. L'autre partie du projet consiste en la mise en évidence des produits au niveau des organes par différentes approches (étude d'activité et imagerie).

Compétences souhaitées :

Une personne volontaire intéressée par l'évaluation d'approche thérapeutique et la caractérisation de l'activité toxique et pharmacologique *in vivo*. Des connaissances anatomo-physiologiques de la souris sont les seules indispensables pour ce projet car le candidat pourra être formé en histologie, biochimie et biologie moléculaire.

Possibilité de financement : à discuter mais possibilité dans le cadre d'une ANR qui se termine en décembre 2009.