MASTER BIOLOGY and HEALTH SCIENCE of LILLE

Research Projects 2024-2025

Projects	Supervisors – Research Units	
Cellular Integrative and Translational Neuroscience		
Maternal behavior Programming on the interplay of Oxytocin, Sialylation, and Inflammation and its regulation by the probiotic Lactobacillus	S. MORLEY-FLETCHER. Team GlycoStress - UMR 8576 CNRS «Glycobiologie Structurale et Fonctionelle», Bât C9 Université de Lille – Villeneuve d'Ascq. sara.morley-fletcher@univ-lille.fr	8
Modelling in vitro neuron-astrocyte interactions in the context of Alzheimer's disease	Sophie HALLIEZ, Alzheimer & Tauopathies, UMR-S 1172, LilNCog, Lille. - sophie.halliez@inserm.fr	8
Development of new types of analgesics	Priscille BRODIN. Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 – UMR 8204 - CIIL - priscille.brodin@inserm.fr	9
Characterization of haematoma in an innovative ex vivo model, towards optimization of the management of patients with intracerebral hemorrhage	Annabelle DUPONT, team 2, UMR Inserm 1011 - annabelle.dupont@univ-lille.fr	9
Deciphering the synapto-protective effect of the Alzheimer's genetic risk factor Pyk2	Devrim KILINC - Inserm U1167 - Team: Molecular Determinants of Alzheimer's Disease and Related Disorders - devrim.kilinc@pasteur-lille.fr	10
Diabetes and Cardiovascular diseases		
Circadian regulation of the glycolytic pathway	Philippe LEFEBVRE, Univ. Lille, UMR Inserm 1011 philippe-claude.lefebvre@inserm.fr	12
Endothelium-immune cell cross-talk in metabolic-associated steatohepatitis.	Anna-Rita CANTELMO et David DOMBROWICZ. UMR 1011. Institut Pasteur de Lille, rue du Pr Calmette– anna-rita.cantelmo@univ-lille.fr ; david.dombrowicz@pasteur-lille.fr	12
Role of the mitochondrial protein import machinery in angiogenesis in health and disease	Anna Rita CANTELMO. U1011 - Récepteurs Nucléaires, Maladies Métaboliques et Cardiovasculaires, Institut Pasteur de Lille, Rue du Professeur Calmette. anna-rita.cantelmo@univ-lille.fr	13

The role of ER-mitochondria contact sites (MAM) in GLP-1 secretion by L cells in the human intestinal organoid model	Sophie LESTAVEL. UMR 1011 INSERM, Laboratoire J & K, Pôle Recherche, Faculté de Médecine, Bd Jules Leclercq, Lille. sophie.lestavel@univ-lille.fr	32
Characterization of a tandem duplication involved in a constitutional epimutation of the MLH1 gene using long-read sequencing and induced pluripotent stem cells	Julie LECLERC - Laboratoire CANTHER, UMR9020 CNRS – U1277 Inserm – Université de Lille – CHU de Lille - julie.leclerc@inserm.fr julie.leclerc@chu-lille.fr	32
Impact of glucocorticoids on islet function: role of SRD5A1 as a modulator of glucocorticoids bioavailability	Stéphanie ESPIARD - Translational Research Laboratory for Diabetes, INSERM UMR1190, Faculté de Médecine, Pôle Recherche, Place de Verdun, Lille - stephanie.espiard@univ-lille.fr	33
Characterization of calcium pathways involving TRPM2 channel in therapy resistance of breast cancer cells	Dimitra GKIKA - CANTHER - UMR 9020 CNRS – UMR 1277 Inserm, Équipe « Plasticité cellulaire et Cancer » - dimitra.gkika(@)univ-lille.fr	33

Cellular Integrative and Translational Neuroscience

Titre: **Maternal behavior Programming on the interplay of Oxytocin, Sialylation, and Inflammation and its regulation by the probiotic Lactobacillus**

Supervisor: **S. MORLEY-FLETCHER**. Team GlycoStress - UMR 8576 CNRS « Glycobiologie Structurale et Fonctionelle », Bât C9 Université de Lille – Villeneuve d'Ascq. 03.20.33.6402; sara.morley-fletcher@univ-lille.fr

Maternal behavior programming plays a pivotal role in shaping the long-term health and adaptive responses of offspring to environmental challenges. This intricate process involves several molecular components, including maternal glucocorticoids, oxytocin (OT), sialylation (a major post-translational modification), and immune response/inflammation. The interconnectedness of these factors impacts immune responses and gut microbiome composition. The M2-2024 project aims to uncover the molecular mechanisms governing the brain-gut axis and understand their roles in the pathological programming caused by maternal stress in the adult male and female offspring. Also, it aims to explore the regulatory action of the probiotic *Lactobacillus reuteri* on maternal and offspring health. The research employs a rat model of PeRinatal Stress (PRS), known to result in reduced OT levels, deficient maternal care, increased inflammation, metabolic syndrome and impaired sialylation in adult offspring. The core hypothesis of the M2-2024 project is that maternal behavior programming arises from the intricate relationship between maternal stress-induced inflammation, systemic and central alterations in N-glycan sialylation, and the subsequent impairment of OT signaling and anti-stress pathways in both mothers and their offspring. The project will investigate the male and female PRS offspring whose mothers have been treated or not with *L. reuteri* during postpartum period. We will consider epigenetic regulation and analyze the sialylation and inflammatory crosstalk within the gut-brain-axis. M2-2024 project holds the promise of unveiling critical insights into the mechanisms underpinning pathology induced by early-life stress and pave the way for novel therapeutic strategies to support maternal well-being and pups' development.

METHODOLOGY. Our animal model is the PRS rat, which has undergone comprehensive characterization in terms of maternal behavior and OT-mediated phenotypes. We will investigate male and female PRS offspring in comparison to unstressed controls, with or without maternal treatment with *L. reuteri.* It's worth noting that estrogens play a role in the expression of glycosyltransferases and sialidases, as well as the inflammatory profile and PRS can lead to sex-specific effects and changes in sex hormones. We will provide sialylation analysis and will focus on inflammatory markers (citokines..) in the brain and the gut.

Title: **Modelling in vitro neuron-astrocyte interactions in the context of Alzheimer's disease**

Supervisor: **Sophie HALLIEZ**, Alzheimer & Tauopathies, UMR-S 1172, LilNCog, Lille. 03 20 29 75 53 - sophie.halliez@inserm.fr

Alzheimer's disease (AD) is a neurodegenerative proteinopathy characterized by the abnormal accumulation in the brain of two types of protein aggregates: extracellular β-amyloid deposits and neurofibrillary lesions mainly constituted of abnormal tau protein. Current research works mostly focus on the way these abnormal protein species propagate through the brain and the subsequent synaptic and neuronal loss. However, it is now quite obvious that astrocytes play a pivotal role in the development of AD and other cellular abnormalities are found in the brain of AD patients. Among them is the overexpression of the adenosine A2A receptor by the astrocytes and the neuronal cells. The research project aims to explore the neuron-astrocyte interactions by the prism of the pathological overexpression of the A2A receptor in the astrocytes in the context of AD. To do so, tripartite synapses will be modelled and characterized using microfluidic chambers integrated with microelectrode arrays. The overexpression of the A2A receptor specifically in the astrocytes will be induced in presence or not of abnormal tau and we will assay its effects on the connectivity and the astrocyte function.

Title : **Development of new types of analgesics**

Supervisor : **Priscille BRODIN**. Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 – UMR 8204 - CIIL - Center for Infection and Immunity of Lille - priscille.brodin@inserm.fr

Understanding pain and relieving its symptoms are two crucial scientific and societal challenges, as the arsenal of analgesics is largely insufficient. One solution to address this situation would be to draw inspiration from "analgesic" solutions deployed and preserved by Mother Nature. Our project is based on a natural system identified by our team (Marion et al. 2014 Cell). Following observations made with patients infected by M. ulcerans (the etiological agent of Buruli ulcer) presenting with massive, painless skin ulcers, we discovered a previously undescribed analgesic system. We demonstrate that the absence of pain is caused by an interaction between mycolactone (a bacterial lipid) and neurons. More precisely, we have identified that mycolactone binds to the AT2R receptor, which specifically activates TRAAK-type potassium channels. This mechanism of action is particularly novel and differs from those of currently available analgesics.
The candidate will search for AT2R ligands inducing TRAAK activation. High-throughput screening of molecules from various chemical library collections capable of activating our system has been undertaken ; the candidate will validate the obtained hits and continue their development through a structure-activity approach (SAR). The candidate will be trained in cell culture, preparation of compounds in microplates on a robotic platform, development of fluorescence cellular assays, confocal microscopy acquisitions, automated image analysis, and results formatting.

Title : **Characterization of haematoma in an innovative ex vivo model, towards optimization of the management of patients with intracerebral hemorrhage**

Tutor : **Annabelle DUPONT**, team 2, UMR Inserm 1011 - 03.20.44.48.45 - annabelle.dupont@univ-lille.fr

Intracerebral hemorrhages (ICH) account for 10-20% of strokes and affect 3.5 million people worldwide every year. Only 50% of patients survive, and half of survivors suffer significant handicap. This poor prognosis is due to the lack of effective treatment for ICH. One way to improve prognosis is to increase haematoma evacuation using a fibrinolytic agent. At present, this approach is not very effective and is contraindicated in patients at high risk of hemorrhage. To optimize this approach and offer it to a greater number of patients, we need to know more about the characteristics of these haematoma. The aim of this project is to characterize haematoma using an innovative ex vivo model developed in the laboratory. The haematoma will be prepared using blood from healthy subjects and patients at high risk of ICH (patients on anticoagulants or with hemorrhagic disease). The effect of antidotes and clotting factor concentrates used in ICH will also be studied. Haematoma will be characterized by several approaches: study of the kinetics of formation, of spontaneous retraction over time and of composition by immunostaining (red blood cells, platelets, leucocytes, fibrin, etc.) combined with a 3D fluorescence imaging approach. The permeability of haematoma and the characteristics of the fibrin network will be assessed by scanning electron microscopy coupled with image analysis. The results obtained will be compared between the different groups of patients and controls. This project will provide important information that will ultimately enable to propose fibrinolytic strategies adapted to each patient. This project is part of the TIPITCH project, which aims to radically transform the prognosis of patients with ICH (https://medecine.univ-lille.fr/fileufr3s/user_upload/ufr3s-actualites/2023/recherche/2023-11-28-rhu-laureat-lillois-projet-tipitch-v4.pdf).

Title : **Deciphering the synapto-protective effect of the Alzheimer's genetic risk factor Pyk2**

Director: **Devrim KILINC** - Inserm U1167 – Risk Factors and Molecular Determinants of Aging-related Diseases - Team: Molecular Determinants of Alzheimer's Disease and Related Disorders - devrim.kilinc@pasteur-lille.fr

Chemical synapses of the nervous system form the basis of learning and memory. Their regulation is a key factor in understanding neuropathological processes leading to cognitive decline and dementia. Accordingly, synapse loss due to the disruption of neuronal plasticity mechanisms is an early event in the Alzheimer's disease (AD) pathogenesis (10.1007/s00401-019-02004-0). Pyk2, product of the AD genetic risk factor PTK2B, is a Ca2+-activated non-receptor tyrosine kinase closely associated with the focal adhesion pathway and known to be involved in the regulation of synapses. Pyk2 activation is disrupted by the binding of the toxic oligomers of amyloid-β (Aβ), a main actor in AD pathophysiology, to a particular synaptic receptor complex (10.1074/jbc.M116.720664). Using custom microfluidic co-cultures, we recently demonstrated that postsynaptic Pyk2 overexpression is protective against amyloid-β (Aβ)-induced synaptic toxicity (10.1093/braincomms/fcaa139), supporting in vivo findings from an AD mouse model (10.1016/j.expneurol.2018.05.020). To further dissect the underlying molecular mechanisms leading to this protective effect, we conducted a bulk RNA sequencing using primary neuronal cultures overexpressing wild-type or mutated (constitutively inactive) Pyk2, exposed to cell-secreted, toxic Aβ forms. Pathway enrichment analysis highlighted the involvement of cytoskeletal and cell adhesion molecules, notably, cadherin, as well as the G protein-coupled receptor signaling, in the neuronal transcriptomic response to Aβ-induced synaptic toxicity. In this M2 project, we aim to further dissect these mechanisms via pharmacological and/or genetic interventions, using imaging-based synaptic read-outs and microelectrode array recordings (10.1021/acsbiomaterials.3c00997). Describing the molecular mechanisms underlying the protective effect of Pyk2 may lead to novel therapeutic strategies targeting the amyloid pathology.

Diabetes and Cardiovascular diseases

Title: **The molecular clock in liver fibrosis**

Mentor: **Philippe LEFEBVRE**, U. Lille, UMR Inserm 1011. E-mail : philippe-claude.lefebvre@inserm.fr

Laboratory background: Our laboratory has a longstanding interest in nonalcoholic steatohepatitis (NASH) and liver fibrosis. We identified altered signaling pathways in humans which may be causal in disease progression (see references below).

Scientific background: Non-alcoholic fatty liver disease (NAFLD) is a spectrum of liver dysfunctions detected in its mildest form as the build-up of excess fat in the liver. Intimately linked to obesity and type 2 diabetes, the disease progresses within years towards an inflammatory state (NASH) and eventually induces liver fibrosis, a detrimental excess of extracellular matrix deposition that strongly impacts physical and functional properties of this organ. The major contributors in the fibrogenic response to liver damage are hepatic stellate cells (HSCs). During NASH, HSCs undergo a critical 'activation' process characterized notably by massive extracellular matrix component production.

Project: The circadian clock (CC) is critical in establishing cellular and tissular homeostasis. Timed by zeitgebers such as light and food intake, organs exhibit cyclic expression of CC mRNA transcripts and of proteins which adjust cellular activities to external cues. Our preliminary data suggest that components of the molecular clock participates into HSC activation. This project will investigate further this relationship.

M2 Objectives: Upon completion of his/her training in an environment fostering scientific interactions, the candidate is expected to master basic cellular and molecular biology techniques and essential analysis tools, to be able to apprehend the general purpose of his/her research project, and to acquire written and oral presentation skills.

Key references: Johanns M. et al. (2023) JHEP Rep., 6(1) 100948 ; Berthier A. et al. (2018) PNAS, 115, E11033-E11042 ; Bobowski-Gerard M. et al. (2022) Nat. Comm. 13.-33063-9 ; Lefebvre P. et al. (2017) JCI Insight 2, e92264 ; Vandel J. et al. (2021) Hepatology 73, 920-936.

Title: **Endothelium-immune cell cross-talk in metabolic-associated steatohepatitis.**

Supervisors: **Anna-Rita CANTELMO et David DOMBROWICZ**. UMR 1011. Institut Pasteur de Lille, rue du Pr Calmette, Lille – 0320877967 - anna-rita.cantelmo@univ-lille.fr ; david.dombrowicz@pasteur-lille.fr

Background. Metabolism-associated steatohepatitis (MASH) is a pathology that can progress to cirrhosis and then to hepatacocarcinoma. In the absence of pharmacological treatment, it is the leading cause of liver transplantation in the USA. The presence of inflammatory infiltrates is one of the hallmarks of MASH, and plays an essential role in the progression of the disease.

The hepatic recruitment of immune cells by diapedesis directly depends on the interactions between immune cells and vascular endothelium which acquires a pro-inflammatory phenotype in this context. However, the precise mechanisms of this cross-talk are still not fully understood.

Objective. This project will examine the potential contribution of immune cell-endothelium interactions to the development of MASH, with the ultimate goal of modulating these interactions for therapeutic purposes.

Methods. Endothelium-immune cell interactions will be studied in vitro. Hepatic endothelial cells and blood immune cells will be isolated from mice developing non-alcoholic steatohepatitis (NASH) following a diet enriched in fats, cholesterol and carbohydrates, and from control mice. Phenotypic alterations in endothelium and immune cells will be analyzed by scRNAseq and validated by in vitro cell activation experiments, including analysis of permeability and transendothelial migration.

Keywords. MASH, Endothelium, Immune cells, Metabolism, Inflammation.

Title: **Role of the mitochondrial protein import machinery in angiogenesis in health and disease**

Tutor: **Anna Rita CANTELMO**. U1011 - Récepteurs Nucléaires, Maladies Métaboliques et Cardiovasculaires, Institut Pasteur de Lille, Rue du Professeur Calmette, Lille. 03 20 33 70 78
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Mitochondria exert central functions in bioenergetics, metabolism, and apoptosis. The correct function of these organelles requires the import of > 1000 nucleus-encoded proteins as the mitochondrial genome provides only 13 proteins. A key component of the mitochondrial protein import machinery is the evolutionarily conserved CHCHD4 oxidoreductase that catalyzes the oxidative folding of targeted proteins after they cross the outer mitochondrial membrane. This mechanism is finely tuned and it is affected in disease.
Using a multidisciplinary approach, combining molecular and cellular biology techniques, this project aims at i) studying the role and functional relevance of CHCHD4 in endothelial cells, and ii) characterizing the signaling pathways that impact on the CHCHD4-dependent import pathway in angiogenesis in disease. The working hypothesis is that aberrant activity of this import pathway drives pathological angiogenesis.
The results generated with this project promise to provide unprecedented insights that will be useful for the development of novel therapeutic strategies for a variety of human diseases characterized by dysfunctional vasculature, such as cardiovascular disorders and cancer.

Title: **Role of « ubiquitin-like protein » FAT10 in the dévelopment of hepatic insulin resistance during MASH developement**

Supervisor: **Réjane PAUMELLE-LESTRELIN**. INSERM UMR1011 “Nuclear receptor, metabolic and cardiovascular diseases”, Laboratoire J & K - Faculté de médecine pôle recherché - Lille - 03 20 97 42 09 - rejane.lestrelin@univ-lille.fr

Metabolic associated steatotic liver disease (MASLD) is now considered the hepatic component of metabolic syndrome and is associated with the development of insulin resistance (IR). This IR is defined as the reduction in the cellular and tissue response to insulin and develops following an accumulation of hepatic triglycerides (steatosis) and chronic inflammatory stress, characteristics of metabolic steatohepatitis (MASH), at high risk of rapid progression to cirrhosis. Although there are clear links, the mechanisms contributing to the development of MASH and hepatic IR remain complex and still poorly understood. Interestingly, transcriptomic analysis of liver biopsies from obese patients developing different grades of MASLD showed that FAT10/UBD expression was positively correlated with MASH severity. Modulation of FAT10 expression in human and murine hepatocytes decreases lipid droplet accumulation during MASLD, and FAT10 deficiency in aged mice has been shown to promote insulin sensitivity, suggesting that FAT10 may contribute to the development of hepatic IR. However, no study to date has demonstrated a direct role for FAT10 in the regulation of the insulin signaling pathway and the development of hepatic IR during MASH. In order to better understand the role of FAT10 in the development of IR during MASH, we propose as part of Master 2, 1) to study the role of FAT10 and its mechanism of action in the response hepatocytes to insulin and IR in a context of MASH in vitro, 2) to determine the role of FAT10 in hepatocytes in IR induced in a context of MASH in vivo in mice.

Title: **Study of FAT10/PPARα interaction during NASH development**

Supervisor: **Audrey HELLEBOID**. UMR1011 « Récepteurs nucléaires, Maladies métaboliques et cardiovasculaires. audrey.helleboid@univ-lille.fr

The prevalence of non-alcoholic fatty liver disease (NAFLD) is on the rise. NAFLDs are initiated by steatosis progressing to non-alcoholic steatohepatitis (NASH) characterized by inflammation, ballooning of hepatocytes, and sometimes fibrosis which can progress to more serious stages ranging from cirrhosis to hepatocellular carcinoma. No pharmacological treatment is currently available. The laboratory has shown that the activation of PPARα, a nuclear receptor strongly expressed in the liver, known for its anti-inflammatory, anti-fibrotic effects and for promoting lipid metabolism, is a promising therapeutic strategy. However, the gene expression of PPARα as well as its activity are reduced in the livers of patients with NASH, partly explaining the ineffectiveness of PPAR agonists in clinical studies treating NASH. It is therefore crucial to better understand the mechanisms underlying this modulation of PPARα during the progression of NASH. Transcriptomic analysis of liver biopsies from obese patients showed that the expression of the FAT10 (UBD) gene, an ubiquitin-like protein, increases during the progression of NASH, and is inversely correlated with the expression of PPARα. FAT10 is known to be responsible for FATylation controlling the stability/degradation and activity of various proteins. Thus, FAT10 could interact with PPARα to modulate its activity during NASH. Our preliminary results show that FAT10 interacts with PPARα in hepatocytes during NASH progression in vivo in murine and human NASH liver biopsies and in vitro in HepG2 cells and contributes to inhibit PPARα activity by its agonist, pemafibrate in vitro and in vivo. FAT10 could therefore promote the progression of NASH by inducing the degradation and/or deactivation of PPARα, making any therapeutic strategy targeting PPARα ineffective. The Master 2 project therefore aims to study the FAT10/PPARα interaction during the development of NASH in vitro and in vivo in order to contribute to the identification of a new therapeutic treatment.

Title : **Evaluation of pharmacological therapies for MASH in a new preclinical mouse model of MASLD**

Supervisor : **Fanny LALLOYER**, UMR1011 (Institut Pasteur of Lille – EGID - University of Lille) - +33320877996 - fanny.lalloyer@univ-lille.fr

MASLD (metabolic dysfunction-associated steatotic liver disease) is the most common liver disease in the world, with a prevalence estimated at 25% of the general population, but reaching 80-90% in obese adults and 50-70% in patients with type 2 diabetes. This pathology has now become a veritable global “epidemic” whose incidence continues to increase, in parallel with the growing epidemic of obesity and diabetes. MASLD is characterized in its first stage by an excessive accumulation of fat in the liver, considered as benign steatosis, in the absence of excessive alcohol consumption. During the progression of MASLD, simple steatosis can progress to MASH (Metabolic dysfunction-associated steatohepatitis), diagnosed as a combination of steatosis, inflammation and ballooning of hepatocytes. In the worst cases, liver damage can progress to fibrosis, cirrhosis and hepatocellular carcinoma, which can lead to the death of the patient. Currently, there is no approved therapeutic treatment for patients with MASH, the aggressive form of NAFLD.
In the laboratory, we developed a new mouse model which presents all stages of human MASLD pathology (liver steatosis, inflammation, ballooning and fibrosis) under high fat diet for 12 weeks. The project aims to better understand MASH physiopathology and to test novel therapeutic targets for MASH in this model. Histological, biochemical and molecular analyzes will be carried out on the various technical platforms of the laboratory.

Title : **Characterization of haematoma in an innovative ex vivo model, towards optimization of the management of patients with intracerebral hemorrhage**

Tutor : **Annabelle DUPONT**, team 2, UMR Inserm 1011 - 03.20.44.48.45 - annabelle.dupont@univ-lille.fr

Intracerebral hemorrhages (ICH) account for 10-20% of strokes and affect 3.5 million people worldwide every year. Only 50% of patients survive, and half of survivors suffer significant handicap. This poor prognosis is due to the lack of effective treatment for ICH. One way to improve prognosis is to increase haematoma evacuation using a fibrinolytic agent. At present, this approach is not very effective and is contraindicated in patients at high risk of hemorrhage. To optimize this approach and offer it to a greater number of patients, we need to know more about the characteristics of these haematoma. The aim of this project is to characterize haematoma using an innovative ex vivo model developed in the laboratory. The haematoma will be prepared using blood from healthy subjects and patients at high risk of ICH (patients on anticoagulants or with hemorrhagic disease). The effect of antidotes and clotting factor concentrates used in ICH will also be studied. Haematoma will be characterized by several approaches: study of the kinetics of formation, of spontaneous retraction over time and of composition by immunostaining (red blood cells, platelets, leucocytes, fibrin, etc.) combined with a 3D fluorescence imaging approach. The permeability of haematoma and the characteristics of the fibrin network will be assessed by scanning electron microscopy coupled with image analysis. The results obtained will be compared between the different groups of patients and controls. This project will provide important information that will ultimately enable to propose fibrinolytic strategies adapted to each patient. This project is part of the TIPITCH project, which aims to radically transform the prognosis of patients with ICH (https://medecine.univ-lille.fr/fileufr3s/user_upload/ufr3s-actualites/2023/recherche/2023-11-28-rhu-laureat-lillois-projet-tipitch-v4.pdf).

Title : **Transcriptional control of the hepatocyte response to liver injury by Ubiquitin D**

Supervisor : **Jérôme EECKHOUTE**. INSERM U1011 Récepteurs nucléaires, maladies cardiovasculaires et diabète, Faculté de Médecine de Lille, Pôle Recherche. Boulevard du Professeur Leclerc, Bâtiment J&K, Lille – 03.20.97.42.19 - jerome.eeckhoute@inserm.fr - https://u1011.pasteur-lille.fr/lunite/theme-4-analyse-transcriptionnelle -integree-des-maladies-hepatiques/

The liver is characterized by its regenerative potential allowing to cope with various insults and replenish the mass of functional hepatocytes. However, acute or chronic diseases can nevertheless promote liver dysfunction underlain by altered hepatocyte regeneration potential and function. Alterations to the hepatocyte transcriptome are involved in this process. In this context, we are seeking to define the role of Ubiquitin D (UBD also known as FAT10). FAT10 is a member of the eukaryotic ubiquitin-like protein family, weakly expressed in normal liver but increased by inflammatory signals upon injury. FAT10 contains two UBL domains enabling covalent interaction (FATylation) via ligases (USE1 and UBA6), or non-covalent interaction, leading its substrates to proteasomal or lysosomal degradation. Our laboratory has already developed a panel of tools to assess how FAT10 controls the hepatocyte transcriptional program (e.g. stable cell-line with FAT10 overrexpression).
We propose a Master 2 internship where the goal will be to perform in vitro assays using our already established cell-lines to define how FAT10 overrexpression or silencing impacts on expression/activity of hepatocyte transcription factors. Cellular and molecular biology approaches to define gene expression, protein levels and subcellular localization will be implemented.

Title: **RevErbα in the gut as target in the control of metabolic disorders-related complications**

Tutor: **Olivier BRIAND** – INSERM U1011 – EGID - Laboratoire JK, Pôle recherché, Faculté de médecine, boulevard du Pr Jules Leclercq, Lille - 03 20 97 42 11 – olivier.briand@univ-lille.fr

Nuclear receptors are transcription factors that modulate the expression of target genes in response to specific ligands. Among these, Rev-Erbα is highly expressed in the body and participates in energy homeostasis, coordinating lipid, carbohydrate and bile acid metabolism with the biological clock.
The intestine plays a unique role in metabolic defense, regulating the absorption of dietary lipids and also contributing to glucose homeostasis. As an endocrine organ, it secretes large quantities of hormones and bioactive peptides that regulate various metabolic processes such as energy homeostasis, intestinal motility and local immunity, as well as its barrier function toward the microbiota. Significant abnormalities in intestinal function favor type 2 diabetes and obesity, leading to atherosclerosis and steatohepatitis.
The project we are proposing for an M2 is part of our research into the molecular mechanisms by which the nuclear receptor RevErbα, expressed in intestinal cells, controls specific intestinal functions such as dietary lipid absorption, barrier function or enteroendocrine response. It is based on important preliminary results in the human enterocyte model Caco-2/TC7 and in murine intestinal organoids, showing disruption of dietary lipid metabolism in the absence of RevErbα.
The approaches employed involve cell and molecular biology techniques (gene and protein expression analysis, indirect immunofluorescence and video microscopy, protein half-life, gene invalidation and overexpression, etc.) and the use of omics approaches. This project is based on cell culture work (filter culture of the human enterocyte line Caco-2/TC7 and murine and human intestinal organoids) and the use of animal models.

Title: **The role of ER-mitochondria contact sites (MAM) in GLP-1 secretion by L cells in the human intestinal organoid model**

Supervisor: **Sophie LESTAVEL**. UMR 1011 INSERM (Dir. Bart STAELS) - Laboratoire J&K, Pôle Recherche Faculté de Médecine, Boulevard du Pr Jules Leclercq, Lille. sophie.lestavel@univ-lille.fr

Type 2 diabetes, linked to dysregulation of glucose metabolism, is a global health emergency. In long term, it can lead to cardiometabolic complications. The intestine plays a major endocrine role by secreting hormones including the incretin GLP-1 (Glucagon-Like Peptide 1), which ensures glycaemic balance by potentiating the secretion of insulin by pancreatic β-cell in response to glucose (Lu et al., 2021). The contact sites between the endoplasmic reticulum and mitochondria (MAM: Mitochondria-Associated Membranes) and their dynamics are essential for ensuring insulin sensitivity in liver and muscle and insulin secretion by the pancreas (Rieusset, 2018). Preliminary in vitro results in a murine L cell line show that MAMs are also involved in GLP-1 secretion.
The aim of the M2 internship is to study the role of MAMs in GLP-1 secretion by intestinal L cells using an original and complex ex vivo model of human intestinal organoids. The organoids will be exposed to various GLP-1 secretagogues (glucose, bile acids, fatty acids, amino acids, etc.). GLP-1 will be measured by ELISA in the supernatant and MAMs will be quantified by PLA (Proximity Ligation Assay) specifically in L cells using GLP-1 immunolocalization. These techniques are mastered in the laboratory. Following the development of adenovirus transfection of organoids, MAMs will be inhibited by a protein, the FATE1 spacer, in order to confirm the role of MAMs in GLP-1 secretion by human intestinal epithelium.
These results should help to position MAMs as potential therapeutic targets in type 2 diabetes to restore insulin sensitivity and increase insulin secretion.

Project title: **Impact of glucocorticoids on islet function: role of SRD5A1 as a modulator of glucocorticoids bioavailability**

Tutor: **Stéphanie ESPIARD** - Translational Research Laboratory for Diabetes, INSERM UMR1190, Faculté de Médecine, Pôle Recherche, Place de Verdun, Lille - 03 20 62 69 63 - stephanie.espiard@univ-lille.fr

Glucocorticoids (GCs) play a pivotal role in regulating physiological processes, including glucose and lipid homeostasis. Overexposure to GCs, whether through endogenous cortisol excess or synthetic GC treatments, results in metabolic complications, notably diabetes. The metabolism of GCs within metabolic tissues is crucial in regulating their bioavailability, with the HSD11B1 and SRD5A1 enzymes playing critical roles. Interestingly, in obesity, a state of tissular cortisol overexposure contributing to the onset of metabolic complications has been described. While HSD11B1 has been extensively studied, clinical trials targeting this enzyme have yielded limited benefits. In contrast, SRD5A1's involvement in obesity-induced metabolic complications remains underexplored. Interestingly, recent animal models and human studies suggest that inhibiting SRD5A1 increases the risk of developing metabolic complications, including diabetes. We also postulate that enhancing SRD5A1 activity could mitigate the metabolic dysfunctions associated with excessive GCs exposure, as seen in obesity and synthetic GC therapy.

The master's student project will focus on pancreatic beta-cell function. The aim would be to study, using human islets, the consequences of SRD5A1 overexpression on glucose-stimulated insulin secretion after treatment by GCs. All the experiments necessary for this project are mastered and routinely used in the lab.

Fundamental and clinical oncology

Title : **Role of O-GlcNAcylation in colorectal cancer response to the FOLFOX chemotherapy**

Project tutor : **Ikram EL YAZIDI** – Structural and functional Glycobiology Unit UMR CNRS 8576, University of Lille, Cité scientifique, C9 building, 59655 Villeneuve d'Ascq – +33 320336499 - ikram.el-yazidi@univ-lille.fr

FOLFOX is used as chemotherapy in the treatment of advanced and metastatic colorectal cancer (CRC). A clinical study correlates CRC mortality in Stage III, recurrence after treatment with 5-FU (one of the two FOLFOX drugs) and a high-carbohydrate diet. Other studies show a link between this recurrence and metabolic disorders. Protein O-GlcNAcylation is a post-translational modification considered as a sensor of cellular nutritional status. It is increased in CRC and metabolic disorders. In order to understand the chemoresistance to FOLFOX, in a normal or physiopathological contexts of diabetes and obesity, we propose to decipher the molecular relationships between O-GlcNAcylation and the resistance mechanisms the to FOLFOX therapy.
The project aims to identify the actors regulated by O-GlcNAcylation and involved in the response to FOLFOX through transcriptomic and glyco-proteomic studies. This research will be conducted on normal or cancerous human colon cells, sensitive or resistant to FOLFOX and tumor tissues. For all groups of cells, whether or not treated with O-GlcNAcylation regulators in the presence or not of FOLFOX, mRNA quantifications by real-time Q-PCR for the actors involved in the response to this chemotherapy will be performed. Similarly, the levels and sub-cellular distribution of the corresponding proteins and O-GlcNAcylation will be analysed by Western-blot and immunohistochemistry. The comparison of results obtained in vitro and on tissues will contribute to understand the role of O-GlcNAcylation in chemoresistance mechanisms of colorectal cancer to FOLFOX.

Title: **Role of the mitochondrial protein import machinery in angiogenesis in health and disease**

Tutor: **Anna Rita CANTELMO**. U1011 - Récepteurs Nucléaires, Maladies Métaboliques et Cardiovasculaires, Institut Pasteur de Lille, Rue du Professeur Calmette, Lille. 03 20 33 70 78
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Mitochondria exert central functions in bioenergetics, metabolism, and apoptosis. The correct function of these organelles requires the import of > 1000 nucleus-encoded proteins as the mitochondrial genome provides only 13 proteins. A key component of the mitochondrial protein import machinery is the evolutionarily conserved CHCHD4 oxidoreductase that catalyzes the oxidative folding of targeted proteins after they cross the outer mitochondrial membrane. This mechanism is finely tuned and it is affected in disease.
Using a multidisciplinary approach, combining molecular and cellular biology techniques, this project aims at i) studying the role and functional relevance of CHCHD4 in endothelial cells, and ii) characterizing the signaling pathways that impact on the CHCHD4-dependent import pathway in angiogenesis in disease. The working hypothesis is that aberrant activity of this import pathway drives pathological angiogenesis.
The results generated with this project promise to provide unprecedented insights that will be useful for the development of novel therapeutic strategies for a variety of human diseases characterized by dysfunctional vasculature, such as cardiovascular disorders and cancer.

Title: T**esting in Microfluidics (colon-on-a-chip) the impact of bacteria-host interactions on the human intestinal regulatory T cell pathogenicity**

Supervisor: **Franck HOUSSEAU**, Visiting Professor - PhyCell U1003 Team 2 – ONCOLille Building - fhousse1@jhmi.edu

Background. The mucosal immune cells derived from the interactions of pathobionts such as enterotoxigenic Bacteroides fragilis (ETBF) with the intestinal barrier may promote dysplasia and modulate the efficacy of immunotherapies. However, human investigations are limited by the absence of models mimicking human physiology. We plan therefore to use microfluidics (colon-on-a-chip) to test how ETBF interact with human colonic epithelial cells and subsequently impact the properties of immune cells from the same patient.
Hypothesis. Based on our preliminary data (Geis et al. Cancer Discov 2016; Destefano-Shields et al Cancer Discov 2021), we postulate that interaction between ETBF and hCEC generate inflammatory signaling driving the differentiation of pathogenic regulatory T cells (Treg) responsible for epithelial dysplasia (carcinogenesis) and suppression of the tumor immunosurveillance (therapy resistance). Microfluidics devices will allow to identify in a human set up the epithelial inflammatory signaling and metabolism affecting human Treg.
Goals. Human intestinal barrier and vessels biomimetics will allow us to explore bacterial toxin-triggered intraepithelial signaling and metabolic pathways shaping the pathogenicity of mucosal Treg. Transcriptomics (RNA seq), metabolomics, proteomics (immunofluorescence) and epigenetics (ATAC seq) approaches will be used.
Impact. Detection and characterization of immunometabolic signatures in blood and tumor may provide biomarkers of resistance to treatment, which can guide the prognostic and medical decision.
Key-words: Colorectal cancer, Immunotherapies, Microfluidics, Pathobionts, Toxins.

Title: **Study of inflammasome-dependent mechanisms in chemotherapy-induced neuropathies.**

Supervisor: **Lionel POULIN** - PhyCell U1003 Equipe 2 - Bâtiment ONCOLille - lionel.poulin@cnrs.fr

Background. Chemotherapy treatments, such as Folfirinox and Palcitaxel, are generally accompanied by serious side effects that limit their use. In particular, chemotherapy-induced peripheral neuropathy (CIPN) is characterized by allodynia or thermal (particularly cold) and mechanical hyperalgesia in up to 80% of patients.
Hypothesis. Activation of inflammasome in macrophages that are located in the dorsal root ganglia (DRG) are thought to be involved in the development of CIPN through the secretion of interleukin-1 beta.
Goals. Single-cell transcriptomics data will be available to define the type of macrophages in which inflammasome actiavtion may promote the development of CIPN as well as the type of neurons that are responsive to IL-1beta and IL-18. This work will involve the use of different tools including cell lines and transgenic animals together with techniques such as cytometry, cell culture, ELISA, Westernblot, histology and others.
Impact. This project, funded by the Ligue contre le Cancer, brings together several partners with complementary expertise.
Key-words: Allodynia, Chemotherapy, Inflammasome, Macrophages.

Title: **Characterization of a tandem duplication involved in a constitutional epimutation of the MLH1 gene using long-read sequencing and induced pluripotent stem cells**

Tutrice : **Julie LECLERC** - Laboratoire CANTHER (Hétérogénéité, Plasticité et Résistance des Cancers aux Thérapies), UMR9020 CNRS – U1277 Inserm – Université de Lille – CHU de Lille - julie.leclerc@inserm.fr julie.leclerc@chu-lille.fr

Constitutional epimutations of the MLH1 gene are an alternative mechanism to genetic mutations in the etiology of Lynch syndrome, a cancer predisposition syndrome. Patients with this epigenetic alteration exhibit hypermethylation of the MLH1 promoter. The precise molecular mechanisms underlying this hypermethylation remain poorly understood. While initially considered to be non-transmissible to offspring due to the erasure of epigenetic marks in germ cells, cases of intergenerational transmission have been documented, often linked to the presence of a genetic event in cis (secondary epimutations). We identified specific genetic variants segregating with the hypermethylation in families with secondary epimutations, including a tandem duplication spanning 29.5 kb (Leclerc et al., Genetics in Medicine 2018). The aim of the project is to further characterize this duplication using innovative strategies. (1) Long-read Nanopore sequencing will be used to simultaneously analyze the genetic sequence and methylation profile across the MLH1 gene. This analysis may be complemented by Optical Genome Mapping using Bionano technology. (2) Induced pluripotent stem cells (iPSCs) have been generated from differentiated cells of a patient carrying the 29.5 kb duplication, thus creating a cellular model of this epimutation. These iPSCs will be used to characterize molecular mechanisms involved in hypermethylation, and to test demethylating therapies (epigenome editing). Additionally, they will be differentiated into organoids for further study.

Key words: epigenetics; oncogenetics; induced pluripotent stem cells; organoids; long-read sequencing.

Title: C**haracterization of calcium pathways involving TRPM2 channel in therapy resistance of breast cancer cells**

Supervisor: **Dimitra GKIKA -** CANTHER « Hétérogénéité, plasticité et résistance aux thérapies des cancers », UMR 9020 CNRS – UMR 1277 Inserm, Équipe « Plasticité cellulaire et Cancer » - dimitra.gkika(@)univ-lille.fr

Ion channels have recently emerged as crucial players in the process of carcinogenesis, presenting themselves as promising therapeutic targets. In our laboratory, we have established the expression profile of TRP channels in triple negative cells after chemo- and radiotherapy treatment and identified TRPM2 as a channel increasing cell viability. The objective of this Master's internship will be to understand how the TRPM2 channel influences cell persistence, focusing particularly on their ability to adapt to chemo- and radiotherapy treatments, thereby contributing to their aggressive nature. Specifically, the candidate student will characterize the calcium signaling pathways promoting the cell viability of persistent cells.

Immunity, Inflammation, Infection

Title: **Understanding the mechanisms governing virulence of the tachyzoite proliferative form of *Toxoplasma gondii***

Supervisor: **Mathieu GISSOT.** Centre d'Infection et d'Immunité de Lille. Institut Pasteur de Lille. 1, rue du Pr. Calmette. 59000 Lille.
mathieu.gissot@pasteur-lille.fr

Toxoplasma gondii is a unicellular eukaryote of the Apicomplexa phylum, which contains many deadly protozoan parasites such as Plasmodium (the cause of malaria) and Cryptosporidium (responsible for cryptosporidiosis). *T. gondii* is of critical importance to pregnant women, with first-time infections having the potential to cause severe illness and even death in the developing fetus. Paramount to the adaptability of *T. gondii* is its complex life cycle, which is characterized by multiple differentiation steps that are essential for its survival in both the human and definitive feline host. However, the molecular mechanisms controlling proliferation are still unknown. Our goal is to understand the mechanisms governing virulence of the tachyzoite proliferative form, a step being the most relevant for pathogenesis in humans. We identified a transcription factor that may be crucial for the parasite survival and proliferation. Specifically, this protein may regulate the formation of daughter cells during the tachyzoite cell cycle. We aim at understanding the role of this regulator in the control of the parasite proliferation. For that, we will use reverse genetics, transcriptomics and proteomics to decipher the biological role of this transcription factor.

Title: **Endothelium-immune cell cross-talk in metabolic-associated steatohepatitis.**

Supervisors: **Anna-Rita CANTELMO et David DOMBROWICZ**. UMR 1011. Institut Pasteur de Lille, rue du Pr Calmette, Lille – 0320877967 - anna-rita.cantelmo@univ-lille.fr ; david.dombrowicz@pasteur-lille.fr

Background. Metabolism-associated steatohepatitis (MASH) is a pathology that can progress to cirrhosis and then to hepatacocarcinoma. In the absence of pharmacological treatment, it is the leading cause of liver transplantation in the USA. The presence of inflammatory infiltrates is one of the hallmarks of MASH, and plays an essential role in the progression of the disease.
The hepatic recruitment of immune cells by diapedesis directly depends on the interactions between immune cells and vascular endothelium which acquires a pro-inflammatory phenotype in this context. However, the precise mechanisms of this cross-talk are still not fully understood.
Objective. This project will examine the potential contribution of immune cell-endothelium interactions to the development of MASH, with the ultimate goal of modulating these interactions for therapeutic purposes.
Methods. Endothelium-immune cell interactions will be studied in vitro. Hepatic endothelial cells and blood immune cells will be isolated from mice developing non-alcoholic steatohepatitis (NASH) following a diet enriched in fats, cholesterol and carbohydrates, and from control mice. Phenotypic alterations in endothelium and immune cells will be analyzed by scRNAseq and validated by in vitro cell activation experiments, including analysis of permeability and transendothelial migration.
Keywords. MASH, Endothelium, Immune cells, Metabolism, Inflammation.

Title: **Improving tuberculosis treatment by targeting the host**

Supervisor: **Priscille BRODIN**. Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 – UMR 8204 - CIIL - Center for Infection and Immunity of Lille - priscille.brodin@inserm.fr

Tuberculosis (TB) remains a pressing public health challenge, exacerbated by the absence of an effective vaccine and the emergence of antibiotic-resistant strains of Mycobacterium tuberculosis (Mtb). Infection with Mtb typically results in chronic pulmonary disease, characterized by severe lung inflammation and pathology. The standard treatment for TB involves a 6-month regimen of four antibiotics, but this duration can extend to up to 2 years with more aggressive therapies for drug-resistant strains, particularly in cases with advanced pathology. Urgent action is needed to develop innovative strategies that not only limit the emergence of resistant strains but also address the complex needs of patients with advanced disease.

Recent advancements have highlighted the potential of host-targeted therapies as complementary approaches to conventional antibiotic treatments. In this context, the focus is on identifying drugs that can enhance the efficacy of antibiotics within the specific environment of Mtb-infected macrophages. High-content screening (HCS) imaging techniques will be employed to precisely quantify antibacterial activity. The candidate will receive comprehensive training in various methodologies, including cell culture techniques, infection experiments, management of compound libraries targeting eukaryotic cells, confocal microscopy procedures, automated image analysis, and the evaluation of synergistic effects resulting from combinations of chemical compounds.

Precision Health

Title: **The molecular clock in liver fibrosis**

Mentor: **Philippe LEFEBVRE**, U. Lille, UMR Inserm 1011. E-mail : philippe-claude.lefebvre@inserm.fr

Laboratory background: Our laboratory has a longstanding interest in nonalcoholic steatohepatitis (NASH) and liver fibrosis. We identified altered signaling pathways in humans which may be causal in disease progression (see references below).

Scientific background: Non-alcoholic fatty liver disease (NAFLD) is a spectrum of liver dysfunctions detected in its mildest form as the build-up of excess fat in the liver. Intimately linked to obesity and type 2 diabetes, the disease progresses within years towards an inflammatory state (NASH) and eventually induces liver fibrosis, a detrimental excess of extracellular matrix deposition that strongly impacts physical and functional properties of this organ. The major contributors in the fibrogenic response to liver damage are hepatic stellate cells (HSCs). During NASH, HSCs undergo a critical 'activation' process characterized notably by massive extracellular matrix component production.

Project: The circadian clock (CC) is critical in establishing cellular and tissular homeostasis. Timed by zeitgebers such as light and food intake, organs exhibit cyclic expression of CC mRNA transcripts and of proteins which adjust cellular activities to external cues. Our preliminary data suggest that components of the molecular clock participates into HSC activation. This project will investigate further this relationship.

M2 Objectives: Upon completion of his/her training in an environment fostering scientific interactions, the candidate is expected to master basic cellular and molecular biology techniques and essential analysis tools, to be able to apprehend the general purpose of his/her research project, and to acquire written and oral presentation skills.

Key references: Johanns M. et al. (2023) JHEP Rep., 6(1) 100948 ; Berthier A. et al. (2018) PNAS, 115, E11033-E11042 ; Bobowski-Gerard M. et al. (2022) Nat. Comm. 13.-33063-9 ; Lefebvre P. et al. (2017) JCI Insight 2, e92264 ; Vandel J. et al. (2021) Hepatology 73, 920-936.

Title : **Role of O-GlcNAcylation in colorectal cancer response to the FOLFOX chemotherapy**

Project tutor : **Ikram EL YAZIDI** – Structural and functional Glycobiology Unit UMR CNRS 8576, University of Lille, Cité scientifique, C9 building, 59655 Villeneuve d'Ascq – +33 320336499 - ikram.el-yazidi@univ-lille.fr

FOLFOX is used as chemotherapy in the treatment of advanced and metastatic colorectal cancer (CRC). A clinical study correlates CRC mortality in Stage III, recurrence after treatment with 5-FU (one of the two FOLFOX drugs) and a high-carbohydrate diet. Other studies show a link between this recurrence and metabolic disorders. Protein O-GlcNAcylation is a post-translational modification considered as a sensor of cellular nutritional status. It is increased in CRC and metabolic disorders. In order to understand the chemoresistance to FOLFOX, in a normal or physiopathological contexts of diabetes and obesity, we propose to decipher the molecular relationships between O-GlcNAcylation and the resistance mechanisms the to FOLFOX therapy.

The project aims to identify the actors regulated by O-GlcNAcylation and involved in the response to FOLFOX through transcriptomic and glyco-proteomic studies. This research will be conducted on normal or cancerous human colon cells, sensitive or resistant to FOLFOX and tumor tissues. For all groups of cells, whether or not treated with O-GlcNAcylation regulators in the presence or not of FOLFOX, mRNA quantifications by real-time Q-PCR for the actors involved in the response to this chemotherapy will be performed. Similarly, the levels and sub-cellular distribution of the corresponding proteins and O-GlcNAcylation will be analysed by Western-blot and immunohistochemistry. The comparison of results obtained in vitro and on tissues will contribute to understand the role of O-GlcNAcylation in chemoresistance mechanisms of colorectal cancer to FOLFOX.

Sujet : **Study of the unfolding protein response (UPR) and glycosylation profile in the brain and plasma of PeRinatal Stress (PRS) model rats and after chronic alcohol consumption as a function of sex differences.**

Tutrice : **Stefania MACCARI**, GlycoStress Team, UGSF, UMR 8576 CNRS, « Glycobiologie Structurale et Fonctionelle » Bât C9 Université de Lille – Campus Scientifique, Villeneuve d'Ascq - stefania.maccari@univ-lille.fr

During pregnancy, stress can affect fetal development via the hypothalamic-pituitary-adrenal (HPA), also leading to reduced maternal care and, consequently, potential dysregulation of fetal brain development and altered expression profiles of many proteins in PeRinatal Stress model (PRS) rats. This could be due to an alteration in the initiation, pruning and elongation mechanisms of N-glycans, which can potentially lead to an unfolded protein response (UPR). No studies have demonstrated a link between altered N-glycan profiling and UPR in adult male and female PRS rats. Our preliminary studies showed an alteration in N-glycan profiles that was partially restored with antidepressant treatment. In particular, an increase in syalisation was observed in the hippocampus of adult male PRS rats. Microarray analysis revealed dysregulation of gene expression, for example of ATF6, which plays a role in UPR, or of CREB3L4, involved in the stress response and apoptosis. Several studies have also established a link between excessive alcohol consumption and deficiencies in N-glycosylation. In this context, the working hypothesis of the M2 project is that PRS and alcohol consumption would impact on the ER N-glycosylation pathway, potentially inducing chronic cellular ER stress. This, in turn, may disrupt the cellular balance of the UPR resulting in neuroreceptors with erroneous glycosylation patterns in rats suffering from PRS and/or alcohol-related conditions. The ultimate goal of this M2 proposal is to pharmacologically target glycosylation to mitigate the effects of PRS on alcohol consumption.

Experimental Protocol. Young-adult male and female rats will be used. The half of rats will be submitted to the PRS protocol and the other half part will be unstressed controls. The same-sex rats are pair housed (2 rats/cage) in their home cages before the start of alcohol intake at adolescence postnatal days (PND), during alcohol consumption rats will be individually housed. We use an alcohol intermittent administration adapted from the Kabbaj alcohol protocol and Dong et al. (2022). We will dispose of 4 groups of rats for males and females, respectively, (CONT/Water, CONT/Alcohol, PRS/Water and PRS/Alcohol for both sexes). Behavioral analysis will be provided as well as tissue (brain, plasma/serum) collection for biochemical, molecular and glycomic analysis.

Title: **Modelling in vitro neuron-astrocyte interactions in the context of Alzheimer's disease**

Supervisor: Sophie HALLIEZ, Alzheimer & Tauopathies, UMR-S 1172, LilNCog, Lille. 03 20 29 75 53 - sophie.halliez@inserm.fr

Alzheimer's disease (AD) is a neurodegenerative proteinopathy characterized by the abnormal accumulation in the brain of two types of protein aggregates: extracellular β-amyloid deposits and neurofibrillary lesions mainly constituted of abnormal tau protein. Current research works mostly focus on the way these abnormal protein species propagate through the brain and the subsequent synaptic and neuronal loss. However, it is now quite obvious that astrocytes play a pivotal role in the development of AD and other cellular abnormalities are found in the brain of AD patients. Among them is the overexpression of the adenosine A2A receptor by the astrocytes and the neuronal cells. The research project aims to explore the neuron-astrocyte interactions by the prism of the pathological overexpression of the A2A receptor in the astrocytes in the context of AD. To do so, tripartite synapses will be modelled and characterized using microfluidic chambers integrated with microelectrode arrays. The overexpression of the A2A receptor specifically in the astrocytes will be induced in presence or not of abnormal tau and we will assay its effects on the connectivity and the astrocyte function.

Title: **Endothelium-immune cell cross-talk in metabolic-associated steatohepatitis.**

Supervisors: **Anna-Rita CANTELMO et David DOMBROWICZ**. UMR 1011. Institut Pasteur de Lille, rue du Pr Calmette, Lille – 0320877967 - anna-rita.cantelmo@univ-lille.fr ; david.dombrowicz@pasteur-lille.fr

Background. Metabolism-associated steatohepatitis (MASH) is a pathology that can progress to cirrhosis and then to hepatacocarcinoma. In the absence of pharmacological treatment, it is the leading cause of liver transplantation in the USA. The presence of inflammatory infiltrates is one of the hallmarks of MASH, and plays an essential role in the progression of the disease.
The hepatic recruitment of immune cells by diapedesis directly depends on the interactions between immune cells and vascular endothelium which acquires a pro-inflammatory phenotype in this context. However, the precise mechanisms of this cross-talk are still not fully understood.
Objective. This project will examine the potential contribution of immune cell-endothelium interactions to the development of MASH, with the ultimate goal of modulating these interactions for therapeutic purposes.
Methods. Endothelium-immune cell interactions will be studied in vitro. Hepatic endothelial cells and blood immune cells will be isolated from mice developing non-alcoholic steatohepatitis (NASH) following a diet enriched in fats, cholesterol and carbohydrates, and from control mice. Phenotypic alterations in endothelium and immune cells will be analyzed by scRNAseq and validated by in vitro cell activation experiments, including analysis of permeability and transendothelial migration.
Keywords. MASH, Endothelium, Immune cells, Metabolism, Inflammation.

Title: **Role of « ubiquitin-like protein » FAT10 in the dévelopment of hepatic insulin resistance during MASH developement**

Supervisor: **Réjane PAUMELLE-LESTRELIN**. INSERM UMR1011 “Nuclear receptor, metabolic and cardiovascular diseases”, Laboratoire J & K - Faculté de médecine pôle recherché - Lille - 03 20 97 42 09 - rejane.lestrelin@univ-lille.fr

Metabolic associated steatotic liver disease (MASLD) is now considered the hepatic component of metabolic syndrome and is associated with the development of insulin resistance (IR). This IR is defined as the reduction in the cellular and tissue response to insulin and develops following an accumulation of hepatic triglycerides (steatosis) and chronic inflammatory stress, characteristics of metabolic steatohepatitis (MASH), at high risk of rapid progression to cirrhosis. Although there are clear links, the mechanisms contributing to the development of MASH and hepatic IR remain complex and still poorly understood. Interestingly, transcriptomic analysis of liver biopsies from obese patients developing different grades of MASLD showed that FAT10/UBD expression was positively correlated with MASH severity. Modulation of FAT10 expression in human and murine hepatocytes decreases lipid droplet accumulation during MASLD, and FAT10 deficiency in aged mice has been shown to promote insulin sensitivity, suggesting that FAT10 may contribute to the development of hepatic IR. However, no study to date has demonstrated a direct role for FAT10 in the regulation of the insulin signaling pathway and the development of hepatic IR during MASH. In order to better understand the role of FAT10 in the development of IR during MASH, we propose as part of Master 2, 1) to study the role of FAT10 and its mechanism of action in the response hepatocytes to insulin and IR in a context of MASH in vitro, 2) to determine the role of FAT10 in hepatocytes in IR induced in a context of MASH in vivo in mice.

Title: **Study of FAT10/PPARα interaction during NASH development**

Supervisor: **Audrey HELLEBOID**. UMR1011 « Récepteurs nucléaires, Maladies métaboliques et cardiovasculaires. audrey.helleboid@univ-lille.fr

The prevalence of non-alcoholic fatty liver disease (NAFLD) is on the rise. NAFLDs are initiated by steatosis progressing to non-alcoholic steatohepatitis (NASH) characterized by inflammation, ballooning of hepatocytes, and sometimes fibrosis which can progress to more serious stages ranging from cirrhosis to hepatocellular carcinoma. No pharmacological treatment is currently available. The laboratory has shown that the activation of PPARα, a nuclear receptor strongly expressed in the liver, known for its anti-inflammatory, anti-fibrotic effects and for promoting lipid metabolism, is a promising therapeutic strategy. However, the gene expression of PPARα as well as its activity are reduced in the livers of patients with NASH, partly explaining the ineffectiveness of PPAR agonists in clinical studies treating NASH. It is therefore crucial to better understand the mechanisms underlying this modulation of PPARα during the progression of NASH. Transcriptomic analysis of liver biopsies from obese patients showed that the expression of the FAT10 (UBD) gene, an ubiquitin-like protein, increases during the progression of NASH, and is inversely correlated with the expression of PPARα. FAT10 is known to be responsible for FATylation controlling the stability/degradation and activity of various proteins. Thus, FAT10 could interact with PPARα to modulate its activity during NASH. Our preliminary results show that FAT10 interacts with PPARα in hepatocytes during NASH progression in vivo in murine and human NASH liver biopsies and in vitro in HepG2 cells and contributes to inhibit PPARα activity by its agonist, pemafibrate in vitro and in vivo. FAT10 could therefore promote the progression of NASH by inducing the degradation and/or deactivation of PPARα, making any therapeutic strategy targeting PPARα ineffective. The Master 2 project therefore aims to study the FAT10/PPARα interaction during the development of NASH in vitro and in vivo in order to contribute to the identification of a new therapeutic treatment.

Title : **Evaluation of pharmacological therapies for MASH in a new preclinical mouse model of MASLD**

Supervisor : **Fanny LALLOYER**, UMR1011 (Institut Pasteur of Lille – EGID - University of Lille) - +33320877996 - fanny.lalloyer@univ-lille.fr

MASLD (metabolic dysfunction-associated steatotic liver disease) is the most common liver disease in the world, with a prevalence estimated at 25% of the general population, but reaching 80-90% in obese adults and 50-70% in patients with type 2 diabetes. This pathology has now become a veritable global “epidemic” whose incidence continues to increase, in parallel with the growing epidemic of obesity and diabetes. MASLD is characterized in its first stage by an excessive accumulation of fat in the liver, considered as benign steatosis, in the absence of excessive alcohol consumption. During the progression of MASLD, simple steatosis can progress to MASH (Metabolic dysfunction-associated steatohepatitis), diagnosed as a combination of steatosis, inflammation and ballooning of hepatocytes. In the worst cases, liver damage can progress to fibrosis, cirrhosis and hepatocellular carcinoma, which can lead to the death of the patient. Currently, there is no approved therapeutic treatment for patients with MASH, the aggressive form of NAFLD.
In the laboratory, we developed a new mouse model which presents all stages of human MASLD pathology (liver steatosis, inflammation, ballooning and fibrosis) under high fat diet for 12 weeks. The project aims to better understand MASH physiopathology and to test novel therapeutic targets for MASH in this model. Histological, biochemical and molecular analyzes will be carried out on the various technical platforms of the laboratory.

Title : **Characterization of haematoma in an innovative ex vivo model, towards optimization of the management of patients with intracerebral hemorrhage**

Tutor : **Annabelle DUPONT**, team 2, UMR Inserm 1011 - 03.20.44.48.45 - annabelle.dupont@univ-lille.fr

Intracerebral hemorrhages (ICH) account for 10-20% of strokes and affect 3.5 million people worldwide every year. Only 50% of patients survive, and half of survivors suffer significant handicap. This poor prognosis is due to the lack of effective treatment for ICH. One way to improve prognosis is to increase haematoma evacuation using a fibrinolytic agent. At present, this approach is not very effective and is contraindicated in patients at high risk of hemorrhage. To optimize this approach and offer it to a greater number of patients, we need to know more about the characteristics of these haematoma. The aim of this project is to characterize haematoma using an innovative ex vivo model developed in the laboratory. The haematoma will be prepared using blood from healthy subjects and patients at high risk of ICH (patients on anticoagulants or with hemorrhagic disease). The effect of antidotes and clotting factor concentrates used in ICH will also be studied. Haematoma will be characterized by several approaches: study of the kinetics of formation, of spontaneous retraction over time and of composition by immunostaining (red blood cells, platelets, leucocytes, fibrin, etc.) combined with a 3D fluorescence imaging approach. The permeability of haematoma and the characteristics of the fibrin network will be assessed by scanning electron microscopy coupled with image analysis. The results obtained will be compared between the different groups of patients and controls. This project will provide important information that will ultimately enable to propose fibrinolytic strategies adapted to each patient. This project is part of the TIPITCH project, which aims to radically transform the prognosis of patients with ICH (https://medecine.univ-lille.fr/fileufr3s/user_upload/ufr3s-actualites/2023/recherche/2023-11-28-rhu-laureat-lillois-projet-tipitch-v4.pdf).

Title : **Insights into ER N-glycosylation via Dolichol metabolism regulation**

Supervisor : **François FOULQUIER**, Unité de Glycobiologie Structurale et Fonctionnelle UMR 8576 CNRS, Univ Lille - 03-20-33-72-58 - francois.foulquier@univ-lille.fr

Dolichol is a very long lipid essential in ER glycosylation pathways such as N-glycosylation, O-/C-mannosylation and GPI anchor synthesis. Dolichol will not only anchor the oligosaccharide that will be transferred en bloc onto nascent glycoproteins but will serve in its monophosphate form as a monosaccharide donor (Dol-P-Man and Dol-P-Glc). Dolichol availability is thus considered as a rate-limiting factor for N-glycosylation and its regulation is absolutely essential. We recently discovered a completely novel metabolic pathway in human dolichol synthesis when deficient leads to a rare glycosylation disease named CDG (Wilson et al., Cell, Accepted). This discovery prompts us to reinvestigate more into details the dolichol synthesis, its recycling and its degradation and how defects in such metabolism can impact ER glycosylation processes. This project aims at (1)_ deciphering the mechanisms by which Dolichol synthesis defects lead to strong glycosylation abnormalities, (2)_ understanding the molecular switch between de novo synthesis and recycling, (3)_ testing the capacity of polyisoprenoid species to compensate for the observed glycosylation defects and lastly, explore the interconnection between cholesterol, dolichol metabolism and glycosylation. This project will use yeasts and mammalian isogenic KO cells.

Title : **Transcriptional control of the hepatocyte response to liver injury by Ubiquitin D**

Supervisor : **Jérôme EECKHOUTE**. INSERM U1011 Récepteurs nucléaires, maladies cardiovasculaires et diabète, Faculté de Médecine de Lille, Pôle Recherche. Boulevard du Professeur Leclerc, Bâtiment J&K, Lille – 03.20.97.42.19 - jerome.eeckhoute@inserm.fr - https://u1011.pasteur-lille.fr/lunite/theme-4-analyse-transcriptionnelle -integree-des-maladies-hepatiques/

The liver is characterized by its regenerative potential allowing to cope with various insults and replenish the mass of functional hepatocytes. However, acute or chronic diseases can nevertheless promote liver dysfunction underlain by altered hepatocyte regeneration potential and function. Alterations to the hepatocyte transcriptome are involved in this process. In this context, we are seeking to define the role of Ubiquitin D (UBD also known as FAT10). FAT10 is a member of the eukaryotic ubiquitin-like protein family, weakly expressed in normal liver but increased by inflammatory signals upon injury. FAT10 contains two UBL domains enabling covalent interaction (FATylation) via ligases (USE1 and UBA6), or non-covalent interaction, leading its substrates to proteasomal or lysosomal degradation. Our laboratory has already developed a panel of tools to assess how FAT10 controls the hepatocyte transcriptional program (e.g. stable cell-line with FAT10 overrexpression).
We propose a Master 2 internship where the goal will be to perform in vitro assays using our already established cell-lines to define how FAT10 overrexpression or silencing impacts on expression/activity of hepatocyte transcription factors. Cellular and molecular biology approaches to define gene expression, protein levels and subcellular localization will be implemented.

Title: **RevErbα in the gut as target in the control of metabolic disorders-related complications**

Tutor: **Olivier BRIAND** – INSERM U1011 – EGID - Laboratoire JK, Pôle recherché, Faculté de médecine, boulevard du Pr Jules Leclercq, Lille - 03 20 97 42 11 – olivier.briand@univ-lille.fr

Nuclear receptors are transcription factors that modulate the expression of target genes in response to specific ligands. Among these, Rev-Erbα is highly expressed in the body and participates in energy homeostasis, coordinating lipid, carbohydrate and bile acid metabolism with the biological clock.
The intestine plays a unique role in metabolic defense, regulating the absorption of dietary lipids and also contributing to glucose homeostasis. As an endocrine organ, it secretes large quantities of hormones and bioactive peptides that regulate various metabolic processes such as energy homeostasis, intestinal motility and local immunity, as well as its barrier function toward the microbiota. Significant abnormalities in intestinal function favor type 2 diabetes and obesity, leading to atherosclerosis and steatohepatitis.
The project we are proposing for an M2 is part of our research into the molecular mechanisms by which the nuclear receptor RevErbα, expressed in intestinal cells, controls specific intestinal functions such as dietary lipid absorption, barrier function or enteroendocrine response. It is based on important preliminary results in the human enterocyte model Caco-2/TC7 and in murine intestinal organoids, showing disruption of dietary lipid metabolism in the absence of RevErbα.
The approaches employed involve cell and molecular biology techniques (gene and protein expression analysis, indirect immunofluorescence and video microscopy, protein half-life, gene invalidation and overexpression, etc.) and the use of omics approaches. This project is based on cell culture work (filter culture of the human enterocyte line Caco-2/TC7 and murine and human intestinal organoids) and the use of animal models.

Title: **The role of ER-mitochondria contact sites (MAM) in GLP-1 secretion by L cells in the human intestinal organoid model**

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Type 2 diabetes, linked to dysregulation of glucose metabolism, is a global health emergency. In long term, it can lead to cardiometabolic complications. The intestine plays a major endocrine role by secreting hormones including the incretin GLP-1 (Glucagon-Like Peptide 1), which ensures glycaemic balance by potentiating the secretion of insulin by pancreatic β-cell in response to glucose (Lu et al., 2021). The contact sites between the endoplasmic reticulum and mitochondria (MAM: Mitochondria-Associated Membranes) and their dynamics are essential for ensuring insulin sensitivity in liver and muscle and insulin secretion by the pancreas (Rieusset, 2018). Preliminary in vitro results in a murine L cell line show that MAMs are also involved in GLP-1 secretion.
The aim of the M2 internship is to study the role of MAMs in GLP-1 secretion by intestinal L cells using an original and complex ex vivo model of human intestinal organoids. The organoids will be exposed to various GLP-1 secretagogues (glucose, bile acids, fatty acids, amino acids, etc.). GLP-1 will be measured by ELISA in the supernatant and MAMs will be quantified by PLA (Proximity Ligation Assay) specifically in L cells using GLP-1 immunolocalization. These techniques are mastered in the laboratory. Following the development of adenovirus transfection of organoids, MAMs will be inhibited by a protein, the FATE1 spacer, in order to confirm the role of MAMs in GLP-1 secretion by human intestinal epithelium.
These results should help to position MAMs as potential therapeutic targets in type 2 diabetes to restore insulin sensitivity and increase insulin secretion.

Title: **Characterization of a tandem duplication involved in a constitutional epimutation of the MLH1 gene using long-read sequencing and induced pluripotent stem cells**

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Constitutional epimutations of the MLH1 gene are an alternative mechanism to genetic mutations in the etiology of Lynch syndrome, a cancer predisposition syndrome. Patients with this epigenetic alteration exhibit hypermethylation of the MLH1 promoter. The precise molecular mechanisms underlying this hypermethylation remain poorly understood. While initially considered to be non-transmissible to offspring due to the erasure of epigenetic marks in germ cells, cases of intergenerational transmission have been documented, often linked to the presence of a genetic event in cis (secondary epimutations). We identified specific genetic variants segregating with the hypermethylation in families with secondary epimutations, including a tandem duplication spanning 29.5 kb (Leclerc et al., Genetics in Medicine 2018). The aim of the project is to further characterize this duplication using innovative strategies. (1) Long-read Nanopore sequencing will be used to simultaneously analyze the genetic sequence and methylation profile across the MLH1 gene. This analysis may be complemented by Optical Genome Mapping using Bionano technology. (2) Induced pluripotent stem cells (iPSCs) have been generated from differentiated cells of a patient carrying the 29.5 kb duplication, thus creating a cellular model of this epimutation. These iPSCs will be used to characterize molecular mechanisms involved in hypermethylation, and to test demethylating therapies (epigenome editing). Additionally, they will be differentiated into organoids for further study.
Key words: epigenetics; oncogenetics; induced pluripotent stem cells; organoids; long-read sequencing.

Project title: **Impact of glucocorticoids on islet function: role of SRD5A1 as a modulator of glucocorticoids bioavailability**

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Glucocorticoids (GCs) play a pivotal role in regulating physiological processes, including glucose and lipid homeostasis. Overexposure to GCs, whether through endogenous cortisol excess or synthetic GC treatments, results in metabolic complications, notably diabetes. The metabolism of GCs within metabolic tissues is crucial in regulating their bioavailability, with the HSD11B1 and SRD5A1 enzymes playing critical roles. Interestingly, in obesity, a state of tissular cortisol overexposure contributing to the onset of metabolic complications has been described. While HSD11B1 has been extensively studied, clinical trials targeting this enzyme have yielded limited benefits. In contrast, SRD5A1's involvement in obesity-induced metabolic complications remains underexplored. Interestingly, recent animal models and human studies suggest that inhibiting SRD5A1 increases the risk of developing metabolic complications, including diabetes. We also postulate that enhancing SRD5A1 activity could mitigate the metabolic dysfunctions associated with excessive GCs exposure, as seen in obesity and synthetic GC therapy.
The master's student project will focus on pancreatic beta-cell function. The aim would be to study, using human islets, the consequences of SRD5A1 overexpression on glucose-stimulated insulin secretion after treatment by GCs. All the experiments necessary for this project are mastered and routinely used in the lab.

Title: C**haracterization of calcium pathways involving TRPM2 channel in therapy resistance of breast cancer cells**

Supervisor: **Dimitra GKIKA -** CANTHER « Hétérogénéité, plasticité et résistance aux thérapies des cancers », UMR 9020 CNRS – UMR 1277 Inserm, Équipe « Plasticité cellulaire et Cancer » - dimitra.gkika(@)univ-lille.fr

Ion channels have recently emerged as crucial players in the process of carcinogenesis, presenting themselves as promising therapeutic targets. In our laboratory, we have established the expression profile of TRP channels in triple negative cells after chemo- and radiotherapy treatment and identified TRPM2 as a channel increasing cell viability. The objective of this Master's internship will be to understand how the TRPM2 channel influences cell persistence, focusing particularly on their ability to adapt to chemo- and radiotherapy treatments, thereby contributing to their aggressive nature. Specifically, the candidate student will characterize the calcium signaling pathways promoting the cell viability of persistent cells.