MASTER BIOLOGY and HEALTH SCIENCE of LILLE

Research Projects 2025-2026

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Cellular Integrative and Translational Neuroscience

Title : **Alpha-synuclein and Acyl-CoA Synthetase Long Chain Family Member 4 in ferroptotic neuronal death in parkinsonian models and patients : translational search for therapeutic targets and biomarkers**

Supervisors : **David DEVOS, Jean-Christophe DEVEDJIAN, Anne-Sophie ROLLAND.** TEAM INSERM 1172 TREAT. tel : 0320445449 david.devos@chu-lille.fr

Parkinson's disease (PD) is characterized by degeneration of dopaminergic neurons associated with aggregation of alpha-synuclein (a-syn) and accumulation of iron in the substantia nigra. We have shown that ferroptosis, a form of regulated death characterized by iron-dependent lipid peroxidation, prevails in the death of dopaminergic neurons. Combined with iron supplementation, natural substrates of Acyl-CoA Synthetase Long Chain Family Member 4 (ACSL4) such as Arachidonic Acid (AA) induce ferroptosis very effectively and specifically. Inhibition of ACS4 has a powerful neuroprotective effect at the cellular level but remains to be demonstrated in in vivo models. Furthermore, the level of a-syn in dopaminergic neurons has a direct impact on the regulation of ferroptosis : suppression of a-syn expression is neuroprotective against pro-ferroptotic agents, whereas overexpression makes neurons more vulnerable.
The aim of this project is to determine whether a-syn modulates ferroptosis via ACSL4, via a direct or indirect relationship. We will conduct this study in vitro on cultured DA neurons (LUHMES dopaminergic neurons) and in vivo in DA-ACSL4 mice (KO of ACSL4 in DA neurons due to Cre-DAT/Lox-ACSL4 transgenes). We will also use large cohorts of patients to analyze at the genetic, epigenetic and protein levels whether ACSL4 activity could be a predictive factor. These results will enable us to develop biomarkers and support a therapeutic program using ACSL4 inhibitors as neuroprotective treatments.

Title: **Effect of a new acellular biotherapy of platelet origin (iN-HPPL) on dopaminergic neurons by examining the involvement of the MAPK and NRF2 pathways in neuroprotective potential.**

Supervisors : **David DEVOS, Jean-Christophe DEVEDJIAN, Anne-Sophie ROLLAND.** TEAM INSERM 1172 TREAT. tel : 0320445449 david.devos@chu-lille.fr

Neurodegenerative diseases affect millions of people worldwide, and there is no treatment that can significantly slow the progression of disability. Our team has developed a new acellular biotherapy based on blood platelets. In addition to their coagulation role, platelets contain the human body's natural repair system. We have patented this treatment, called Human Heat Platelet Pelet with intercept and nanofiltration (iN-HPPL), which is a preparation based on platelet derivatives from healthy donors. It is an abundant source of trophic factors (growth factors, neurotransmitters, neuromodulators, anti-inflammatory and antioxidant proteins, etc.) that regulate the development, maintenance, function and plasticity of the central nervous system. Preclinical results obtained in various models of neurodegeneration (Parkinson's, ALS, head trauma, etc.) have demonstrated iN-HPPL's ability to target the various signaling pathways (oxidative stress, neuroinflammation, cell survival, etc.) that lead to neurodegeneration, in particular AKT/MEK. iN-HPPL also has a powerful anti-ferroptotic action. Two major regulatory pathways, MAPK and NRF2, are implicated in models of neurodegenerative disease. We therefore want to study whether these major transduction pathways are involved in a dependent or independent manner in the neuroprotection of iN-HPPL in a Parkinson's cell model. We will also establish whether this level of neuroprotection is more dependent on exposure time or HPPL concentration and to what extent.

Title: **Exploring the Potential of Oxytocin Therapy to Enhance Brain Connectivity in a Mouse Model of Prader-Willi Syndrome**

Mentor: **Sébastien G. BOURET**, Inserm UMR-S 1172, Development and Plasticity of the Neuroendocrine Brain, Lille Neuroscience & Cognition Research Center. Tel: 03-5950-7551. sebastien.bouret@inserm.fr

Prader-Willi Syndrome (PWS) is a rare genetic disorder caused by an abnormality in chromosome 15, affecting approximately 1 in 20,000 to 25,000 births. The main characteristics of its pathophysiology include severe hypotonia, feeding and sucking difficulties, and growth retardation from birth to early childhood, followed by hyperphagia, risk of morbid obesity, hypogonadism, learning difficulties, moderate intellectual disability, and behavioral issues, particularly in social interactions. A new mouse model for PWS, called the Del Ndn-Magel2 mouse, has been developed with a combined mutation of the Magel2 and Necdin genes. These mice exhibit many disorders similar to those observed in PWS patients. A comparative transcriptomic analysis revealed dysregulation of the glutamatergic system in the hypothalamus of Del Ndn-Magel2 mice and PWS patients. Glutamate is an important neurotransmitter in the central nervous system. We also found a loss of oxytocin production in this mouse model, similar to what has been described in patients with PWS. Interestingly, we recently demonstrated that oxytocin can act as a neurodevelopmental factor to promote the development of hypothalamic melanocortinergic circuits. The objectives of this Master 2 project are to 1) characterize whether glutamatergic neurons in the hypothalamus express oxytocin receptors and if this expression is affected in the context of PWS, 2) explore whether oxytocin treatment during various life periods (i.e., neonatal life, puberty, and adult life) can correct the abnormal glutamatergic inputs observed in Del Ndn-Magel2 mice. This project will provide novel insights into the neurobiological substrates underlying PWS and could open new therapeutic avenues.

Title: **Evaluation of a New Neurotrophic Factor, VGF, as a Pharmacological Target and Modulator of Amyloid Precursor Protein (APP) Metabolism in Alzheimer's Disease**

Supervisor: **Nicolas SERGEANT**. Inserm UMRS 1172, Lille Neuroscience et Cognition, Équipe Innovation Thérapeutique pour le traitement des maladies neurodégénératives (iT4BD). Phone: +33 (0)663101728 - nicolas.sergeant@inserm.fr

Alzheimer's disease is characterized by two main pathological processes in the brain : amyloid deposits and neurofibrillary degeneration (NFD), whose main components are microtubuleassociated Tau proteins and the β-amyloid peptide, which is derived from its precursor, APP. We have developed drug candidates that act on both aspects of the disease. The newly discovered target of our compounds is the neurosecretory and neurotrophic factor VGF, whose loss of function has been established in Alzheimer's disease. We hypothesize that our drug candidates exert their effect through a gain-of-function mechanism of the VGF factor. Thus, it is essential to define the mechanism of this gain-of-function (expression, secretion, signaling, etc.) and the role of VGF in APP metabolism, as our compounds reduce the formation of β-amyloid peptides and influence APP metabolism. Establishing a causal relationship, we hypothesize that VGF directly modulates APP metabolism.
Using cellular and molecular biology approaches, this project aims to: - Define the mechanism of action of our drug candidates on VGF - Determine how VGF modulates the metabolism of the amyloid precursor protein (APP) and Ab peptides production.
This project will involve various techniques, including - Cell Biology : Cell culture and differentiation - Molecular Biology : Transfection, gene expression analysis, siRNA, PCR, and RT-PCR - Microscopy : Immuno-fluorescence imaging - Biochemistry : Western blot and ELISA assays.
Using molecular tools and small pharmacological molecules targeting VGF, the study aims to : - Establish the link between VGF and APP metabolism - Define how our drug candidates modulate this activity.

Project title: **Probing mechanotransduction downstream of synaptic cell adhesion molecules**

Supervisor: **Devrim KILINC** - Inserm U1167 – Team: Risk Factors and Molecular Determinants of Aging-related Diseases - 03 20 87 78 01 - 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). Synapses undergo activity-dependent structural change, which involves a number of mechanically relevant processes, including cytoskeleton and cell adhesion molecules (CAMs) (doi.org/10.3389/fncel.2018.00483). However, little is known about the mechanical aspects of synaptic plasticity, and if and how AD genetic risk factors are involved therein. Within this framework, this M2 project aims to study the role of mechanotransduction downstream of synaptic CAMs in human induced neurons (hiNs). We will induce pre- and post-synapse formation on axons and dendrites through mechanical stimulation of N-Cadherin (NCad), a transsynaptic CAM, via magnetic tweezers force application in microfluidic devices (doi.org/10.1002/adhm.201600895) that fluidically isolate axons and dendrites. Shape change and synaptic protein accumulation will be evaluated via live-cell imaging and immunocytochemistry. In complementary experiments, we will analyze the synaptic localization of NCad as a function of synaptic potentitation and AD-related synaptotoxicity (doi.org/10.1093/braincomms/fcaa139/5898625). This is an ambitious project at the intersection of neurodegenerative diseases and mechanobiology fields that deals with an emerging, yet understudied concept using custom, innovative tools.

Diabetes and Cardiovascular diseases

Title: **PPARα and metabolic memory in diabetic retinopathy**

Tutors: **Anna Rita CANTELMO, David DOMBROWICZ.** U1011 - Récepteurs Nucléaires, Maladies Métaboliques et Cardiovasculaires, Institut Pasteur de Lille, Rue du Professeur Calmette, Lille - Tel: +33 320 87 71 48. anna-rita.cantelmo@univ-lille.fr

Diabetes mellitus affects an increasing global population, with diabetic retinopathy (DR) and diabetic macular edema (DME) being major causes of vision loss. Endothelial dysfunction plays a pivotal role in DR, where chronic hyperglycemia drives persistent metabolic and epigenetic alterations, contributing to 'metabolic memory' and disease progression.

Peroxisome Proliferator-Activated Receptor α (PPARα) serves as a key regulator at the intersection of metabolism and epigenetics. While primarily involved in lipid metabolism, PPARα also modulates inflammatory responses and influences DNA methylation. In diabetes, its expression is repressed due to hypermethylation, which may contribute to sustained endothelial dysfunction. Notably, PPARα agonists have demonstrated protective effects in DR, raising the question of whether their benefits stem from epigenetic modulation rather than metabolic regulation alone.

This project aims to: (1) assess endothelial PPARα epigenetic alterations as potential markers of vascular dysfunction in diabetes, and (2) investigate the interplay between metabolic and epigenetic pathways via PPARα in retinal endothelial cells. This study seeks to uncover novel endothelial targets for therapeutic intervention in DR.

Title: **Regulation of angiogenesis by mitochondrial protein import pathway.**

Tutor: **Anna Rita CANTELMO.** U1011 - Récepteurs Nucléaires, Maladies Métaboliques et Cardiovasculaires, Institut Pasteur de Lille, Rue du Prof Calmette, Lille - Tel: 03 20 33 70 78 . anna-rita.cantelmo@univ-lille.fr

Mitochondria play a pivotal role in bioenergetics, metabolism, and apoptosis. Since the mitochondrial genome encodes only 13 proteins, the proper function of these organelles relies on the import of more than 1000 nucleus-encoded proteins. A crucial component of the mitochondrial protein import machinery is the evolutionarily conserved CHCHD4 oxidoreductase, which facilitates the oxidative folding of imported proteins after they after they cross the outer mitochondrial membrane. This finely regulated process is disrupted in disease conditions. This project employs a multidisciplinary approach, integrating molecular and cellular biology techniques, to:

i) Investigate the role and functional significance of CHCHD4 in endothelial cells;

ii) Characterize the signaling pathways influencing the CHCHD4-dependent import pathway in pathological angiogenesis.

The central hypothesis is that dysregulation of this import mechanism contributes to aberrant angiogenesis. Insights from this study may pave the way for novel therapeutic strategies targeting vascular dysfunction in cardiovascular diseases such as atherosclerosis.

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

Supervision: **Sophie LESTAVEL**. UMR 1011 INSERM - Laboratoire J&K, Pôle Recherche Faculté de Médecine, Bd 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.

Title: **Metabolic control of IL-23 production by resident and migratory dendritic cells.**

Supervisor: **David DOMBROWICZ** – UMR 1011. Institut Pasteur de Lille. rue du Pr Calmette, 0320877967 - david.dombrowicz@pasteur-lille.fr

Background. Dendritic cells (DC) are key to the initiation of the adaptive immune response. They capture antigens in peripheral tissues and migrate to draining lymph nodes (LNs). In psoriasis (PSO), migrating IL-23-producing DCs activate T lymphocytes and IL-17 production in LNs. Metabolism controls key DC functions, and fatty acids exacerbate PSO through their effects on DCs, but the metabolic pathways governing these processes are poorly understood.

Aim. This project will investigate how DC metabolism and in particular the pentose phosphate (PPP) and hexosamine biosynthesis (HBP) pathways affect these parameters to induce psoriasis and contribute to their exacerbation in metabolic pathologies.

Methods. Preclinical in vivo and in vitro models, pharmacological inhibitors and gene modifications will be used to study a) the metabolic requirements of IL-23-producing DCs b) metabolic remodeling during exacerbation of psoriasis by a high-fat diet. The study will focus on enzymes controlling glycolysis and PPP respectively: Pfkfb3 and HBP: Gfpt1, Stt3a. Metabolic analyses based on flow cytometry (SCENITH) and transcriptomic analyses by scRNA-seq will be used.

Collaborations. This work will be undertaken in collaboration wtih Dr Stoyan Invanov (LP2M, Nice).

Keywords. DC, Psoriasis, Métabolism, scRNA-seq, bioinformatic, functionnal tests

Title : **Role of CX3CR1+ T lymphocytes in metabolic diseases**

Supervisors. **David DOMBROWICZ, Laurent L'HOMME** – UMR 1011. Institut Pasteur de Lille. Rue du Pr Calmette. 59019 Lille. 0320877967 - david.dombrowicz@pasteur-lille.fr ; laurent.l-homme@inserm.fr

Context. CD4+ and CD8+ T lymphocytes are major players in adaptive immunity and play diverse roles in the development of metabolic diseases such as obesity, type 2 diabetes and metabolic fatty liver. In liver and adipose tissue, a T lymphocyte subpopulation expresses the CX3CR1 receptor, a receptor for the CX3CL1 chemokine involved in adhesion, migration, tissue retention and cell survival. The role of these CD4+ and CD8+ T lymphocyte subpopulations in the development of metabolic diseases is currently unknown.
Aim. In this project, a characterization and functional study of these subpopulations will be carried out.
Methods. The project will be based primarily on mouse models of obesity and steatosis/steatohepatitis (HFD, HFSCD and CDAA diets) and on flow cytometry. Other techniques such as cell culture, ELISA and RT-PCR as well as transcriptomic approaches (RNA-seq and scRNA-seq) will be used. Ultimately, the project should shed light on the role of these CD4+ and CD8+ T lymphocyte subpopulations in the development of metabolic diseases.
Keywords. T lymphocytes, metabolic diseases, MASLD, MASH, fractalkine.

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, 3ème étage OUEST. Faculté de Médecine de Lille, Pôle Recherche, 1, Place de Verdun, Lille. Phone number: 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 SRD5A1 enzymes playing critical roles. Interestingly, in obesity, a state of tissular cortisol overexposure contributing to the onset of metabolic complications has been described. 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. Previous studies using supraphysiological dose of GCs showed that SRD5A1 overexpression can rescue the impact of GCs on glucose stimulated insulin secretion (GSIS). As GCs have been shown to have a synergistic effect on β-cell dysfunction induced by a glucolipotoxic environment, the aim of the project would be to assess how SRD5A1 modulates the impact of physiological dose of GCs on GSIS in both human islets and INS1 cells cultured with medium enriched in glucose and lipids. All the experiments necessary for this project are mastered and routinely used in the lab.

Title: **Therapeutic Potential of SGLT4 Inhibition in Diet-Induced Metabolic Disease**

Supervisor: **Caroline BONNER**, Institut Pasteur de Lille / Inserm U1190 Translation Research of Diabetes - +33-(0)-32-06-23-413 - caroline.bonner@univ-lille.fr

The rising prevalence of obesity and type 2 diabetes (T2D) necessitates novel therapeutic approaches. We propose targeting sodium-glucose cotransporter 4 (SGLT4), which uniquely transports multiple sugars (mannose > glucose > fructose > 1,5-anhydroglucitol > galactose) via a Na+-dependent system with a Km of 2.6 mM. These sugars predominate in Western diets (WD) and exhibit elevated serum concentrations in individuals with obesity.

Our preliminary data with global Sglt4 knockout mice show remarkable protection against diet-induced obesity and T2D after five months of WD exposure. However, this model is limited as SGLT4 inhibition occurs from embryonic stages, whereas therapeutic intervention in humans would begin in adulthood after metabolic disease is established.

To address this critical question - can SGLT4 inhibition reverse already established metabolic disease? - we developed conditional Sglt4 knockout mice (Sglt4LoxP/LoxP). These mice will be crossed with tamoxifen-inducible UBC-CreERT2 mice to allow targeted SGLT4 deletion in adult mice after obesity is established. Crucially, all study groups will be maintained on WD for three months to develop obesity and metabolic dysfunction before inducing knockout, allowing us to determine if SGLT4 inhibition can drive weight loss and metabolic improvements in already obese mice. We will conduct comprehensive metabolic phenotyping, including body weight trajectories, glycosuria assessment, glucose homeostasis, and tissue-specific expression analyses of glucose transporters.

This project offers an opportunity to study a potential therapeutic strategy that could not only prevent but potentially reverse established metabolic disease.

Title: **Role of « ubiquitin-like protein » FAT10 in the hepatocyte suffering during MASH development**

Supervisor: **Réjane PAUMELLE-LESTRELIN**. INSERM-UMR 1011 "Nuclear receptor, metabolic and cardiovascular diseases". Laboratory J & K - Faculty of Medicine, Research Center - Bd Pr Jules Leclerc, Lille - Tel: 03 20 97 42 09 - rejane.lestrelin@univ-lille.fr

Metabolic dysfunction-associated steatotic liver disease (MASLD) affects 1/3 of the general population, which obesity is the main risk factor. MASLDs are characterized by an intrahepatic accumulation of lipids (steatosis) progressing to metabolic dysfunction-associated steatohepatitis (MASH) which can lead to the development of cirrhosis and hepatocellular carcinoma (HCC). To date, few effective medical treatments for MASH are available due to potential resistance to therapy. The disruption of degradation pathways leading to the formation of aggresomes: the Mallory-Denk-Bodies (MDB), and hepatocyte ballooning, the main histological characteristic of hepatocyte suffering, appears to be a potential mediator of the progression of MASH to cirrhosis and HCC. However, molecular mechanisms contributing to their formation are not known. Our transcriptomic analysis of liver biopsies from obese patients with MASH shows that FAT10 expression correlates positively with different histological grades of MASLD and MASH severity such as MDB. Interestingly, FAT10 is a protein of the "ubiquitin-like" family involved in FATylation processes regulating protein degradation, and is induced by inflammation in metabolic tissues. Interestingly, FAT10 plays a role in the formation of MDB induced by the hepatotoxic agent (DDC) in mice, suggesting that FAT10 may play a role in MDB formation during MASH progression. Our project therefore aims to characterize the role of FAT10 in the formation of MDB and hepatocyte suffering during the MASH severity in murine et human cellular models. This project will identify cellular and molecular mechanisms contributing to the hepatocyte suffering associated to MASH development and progression to cirrhosis and may lead to the identification of new therapeutic targets.

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

Supervisors: **Réjane PAUMELLE-LESTRELIN** and **Guillaume LASSAILLY** (U1286). INSERM-UMR1011 "Nuclear receptor, metabolic and cardiovascular diseases". Laboratory J&K - Faculty of Medicine, Research Center-Bd Pr Jules Leclerc, Lille. Tel: 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 clinical relevance of FAT10 expression in hepatocytes associated with type 2 diabetes status in liver biopsies from obese patients with or without MASH.

Title: **Identification of FAT10 interaction partners involved in hepatocyte injury through a proteomic approach**

Supervisor: **Audrey HELLEBOID**. INSERM-UMR1011 "Nuclear receptor, metabolic and cardiovascular diseases". Laboratory J & K - Faculty of Medicine, Research Center - Bd Pr Jules Leclerc, Lille - audrey.helleboid@univ-lille.fr

Metabolic dysfunction-associated steatotic liver disease (MASLD) affects about one-third of the general population, with obesity being the primary risk factor. This condition is characterized by the accumulation of lipids in the liver (steatosis), which can progress to metabolic dysfunction-associated steatohepatitis (MASH). This condition can lead to the development of cirrhosis and hepatocellular carcinoma (HCC). To date, there are few effective pharmacological treatments for MASH, likely due to resistance mechanisms. Disruptions in protein degradation pathways, leading to the formation of Mallory-Denk bodies (MDB) and hepatocyte ballooning, typical markers of liver damage, may play a key role in the progression of MASH to cirrhosis and HCC. Our transcriptomic analyses of liver biopsies from obese patients with MASH reveal that FAT10 expression is positively correlated with MASLD severity. FAT10, a protein belonging to the "ubiquitin-like" family, is involved in FATylation, a process regulating protein degradation, and is induced by inflammation in metabolic tissues. Studies in mice show that FAT10 is involved in the formation of MDB induced by the hepatotoxin DDC, suggesting it may also play a similar role in the progression of MASH. Our project aims to characterize the role of FAT10 in hepatocyte injury during the progression of MASH. Identifying its interaction partners through a proteomic approach will help characterize the molecular pathways involved in liver damage and understand the role of FATylation in this process, with the hope of identifying new therapeutic targets.

Title: **Role of the nuclear receptor Rev-erbα in angiogenesis**

Supervisor: **Benoit POURCET** – Université de Lille, INSERM U1011 Institut Pasteur de Lille CHU Lille EGID – 01 rue du Pr Calmette – 0320877125 - benoit.pourcet@univ-lille.fr

Atherosclerosis is a chronic inflammatory disease of large vessels triggered by the accumulation of cholesterol and leukocytes in the vascular wall. During atherogenesis, vascular wall thickening induces local hypoxia and promotes the vasa vasorum expansion by angiogenesis. These neovessels are however immature and then promote leakage of lipids and leukocytes thus contributing to plaque progression and rupture. The molecular and cellular mechanisms involved in the growth of the perivascular blood network are not known. Reducing its expansion could, however, represent an innovative therapeutic strategy in the treatment of these diseases. Our preliminary data suggest that the nuclear receptor Rev-erb-α controls angiogenesis and intraplaque neovascularization ex vivo and in vivo. This proposal aims to determine the impact of Rev-erb-α in endothelial cells during angiogenesis using in vivo and in vitro approaches. For that purpose, angiogenesis will be assessed in vivo by confocal and light sheet microscopy in endothelial-specific Rev-erbα-/- mice and their control by analyzing the development of the vascular network of newborn retinas. The role of Rev-erb-α on angiogenic processes will then be analyzed in vitro using 3D spheroid models of cell competition. The pathways involved in angiogenesis will be assessed in tissues and cultured cells by WES and RT-qPCR. This M2R proposal aims to determine the impact of Rev-erb-α in angiogenesis during atherosclerosis and to define the molecular and cellular mechanisms involved.

Title: **Evaluation of pharmacological therapies for MASH, liver fibrosis and atherosclerosis in a new preclinical mouse model combining MASLD and atherosclerosis development**

Supervisor: **Fanny LALLOYER**, Inserm UMR 1011 - Institut Pasteur of Lille - University of Lille. Tel : +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 and in conjunction with cardiometabolic risk factors. 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. However, the majority ofMASLD patients die from cardiovascular diseases. Currently, there is only one pharmacological treatment, resmetirom, approved in the USA for MASH, the aggressive form of MASLD.

In the laboratory, we have set up a new mouse model which progressively develops all stages of human MASLD pathology (liver steatosis, inflammation, ballooning and fibrosis) under high fat diet in a short period of time. The project aims to better understand MASH and liver fibrosis physiopathology and to test novel therapeutic targets for MASLD and its consequences on atherosclerosis development in this model. Histological, biochemical and molecular analyzes will be carried out on the various technical platforms of the laboratory.

Fundamental and clinical oncology

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

Project tutor: **Ikram EL YAZIDI** – Structural and functional Glycobiology Unit UMR CNRS 8576, University of Lille, Cité scientifique, C9 building – Villeneuve d'Ascq – Tel: +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. The levels and sub-cellular distribution of the corresponding proteins and O-GlcNAcylation will be analysed by Western-blot and immunohistochemistry. Capillary electrophoresis and mass spectrometry of metabolites extracted from the cells will be performed to analyze the variation in UDP-GlcNAc levels in response to chemotherapy. The comparison of results obtained in vitro and on tissues will contribute to understand the role of O-GlcN.

Title: **Identification of CD81 partners involved in the aggressiveness of acute myeloid leukemia (AML).**

Tutor: **Cyril COUTURIER**, Canther/Oncolille Laboratory, Equipe PROTECT-L, Tel: 06 69 76 08 06 - cyril.couturier@univ-lille.fr

Our team has shown that the level of CD81 surface expression on AML cells impacts patients' prognosis. Elevated CD81 mRNA levels, correlated with protein overexpression on the cell surface, reduce survival. Moreover, at disease relapse, its expression is higher than at the time of diagnosis. In order to gain a better understanding how CD81 affect poor prognosis in AML, leukemic cell lines expressing no, intermediate or high levels of CD81 on their surface were tested in vivo in murine xenotransplanted models: cells with the highest levels of CD81 were more aggressive and more rapidly promoted death by leukemia burden. An antibody directed against CD81 included into an anti-leukemic treatment protocol was beneficial for both leukemias derived from CD81-overexpressing cell models (CDX) and CD81-overexpressing patient-derived cells (PDX). The intracellular parts of CD81 are not involved in the 5A6 antibody effect promoting similar to our studies, where no changes in gene expression have been shown, indicating rather a physical effect by clustering of CD81 partners. The proposed project involves identifying CD81 partners by proximity labeling in cellulo (APEX3 system). Validation of their interaction with CD81 will be done in live cells, using BRET (Bioluminescence Resonance Energy Transfer). In parallel, silencing approaches would allow us to show their implication in the observed effect. The identification of these partners and their implication in the poor prognosis will enable us to better understand the underlying mechanisms involved, which is important to propose novel and highly specific therapeutic solutions.

Sujet : **Identifying the molecular role of inflammasome activation by oxaliplatin in macrophages.**

Tuteur : **Lionel POULIN** – U1003 Equipe 2 – Institut OncoLille, Boulevard du Professeur Jules Leclercq – 0320965269 - lionel.poulin@cnrs.fr

The Chamaillard laboratory focuses on understanding the role of NOD-like receptors in microbial tolerance, host defense, and antitumoral immunity, as well as their deregulation in Crohn's disease and colitis-associated colorectal cancer. Using advanced techniques and complex genetic models, we have made significant contributions to mucosal immunology, including the identification of novel dendritic cell subsets and pathways involved in inflammation-driven carcinogenesis. Oxaliplatin, a widely used chemotherapy agent, is associated with severe side effects, such as peripheral neuropathic pain, which is dependent on macrophage recruitment. Recently, we identified the Nlrp3 inflammasome as a key player in promoting IL-1β production in macrophages following oxaliplatin exposure. Similar to vincristine, another chemotherapy agent known to induce neuropathic pain through inflammasome activation, oxaliplatin's effects appear to involve inflammatory pathways. The primary objective of this Master's project is to characterize the molecular mechanisms underlying the activation of this NOD-like receptor in macrophages. This research will employ a variety of techniques, including cytometry, cell culture, ELISA, Western blot, and immunofluorescence.

Title : **Differentiation of genome-edited induced pluripotent stem cells (cellular models of MLH1 constitutional epimutations) into colonic organoids**

Tutor : **Julie LECLERC** - 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. To explore these molecular mechanisms, we created cellular models of MLH1 constitutional epimutations based on induced pluripotent stem cells (iPSC). We used the CRISPR-Cas9 technology to modify the MLH1 gene and introduce genetic variants identified in epimutation carriers and associated in cis with promoter hypermethylation (secondary epimutations). While these stem cells showed de novo MLH1 methylation with increasing levels, methylation should be stable in differentiated cells.
The aim of the project is to differentiate these genome-edited iPSC into colonic organoids. This will be done in the ORGALille platform (https://orgalille.univ-lille.fr/) where the student will be trained to iPSC culture and organoid differentiation. Further characterization of the organoids will involve (1) study of their methylation profile using dedicated technics (2) immunofluorescent labeling (3) mutational and microsatellite instability analyses.

Key words: Epigenetics; Oncogenetics; induced pluripotent stem cells; organoids; methylation.

Immunity, Inflammation, Infection

Title: **Testing the impact of bacteria-host interactions on the human intestinal regulatory T cell pathogenicity and resistance to cancer immunosurveillance**

Supervisor: **Franck HOUSSEAU**, PHYCELL INSERM U1003, ONCOLILLE, Univ.ersity of Lille. Associate Professor Adjunct in Oncology, Johns Hopkins University, Baltimore, USA. fhousse1@jhmi.edu / +1 571-246-7562

Mucosal immunity at the intestinal barrier plays a critical role in the immune defenses against pathogens but also in shaping immune environments in distant organs associated with wound repair, tumor immune microenvironment (TiME) or response to immunotherapy. In cases of carcinogenesis and/or anti-tumor immune response, the mucosal immune regulatory T cells derived from the interactions of the pathobiont enterotoxigenic Bacteroides fragilis (ETBF) with the intestinal barrier may promote dysplasia and transition to cancer or migrate to the TiME to impact the functions of the immune effector cells and the antitumor immune response. We plan therefore to test how ETBF colonization in murine models of colon tumorigenesis promotes chronic immunosuppressive programs and the resistance to immune T cell checkpoint blockade.

Based on our preliminary data, we postulate that interactions between ETBF and colonic epithelial cells generate inflammatory signaling driving the differentiation of mucosal pathogenic regulatory T cells (Treg) responsible for epithelial dysplasia (carcinogenesis) and suppression of the tumor immunosurveillance (therapy resistance). We will explore bacterial toxin-triggered intraepithelial signaling and metabolic pathways shaping the pathogenicity of mucosal Treg. Transcriptomics (RNA seq) and proteomics (immunofluorescence and flow cytometry) approaches will be used. The ETBF-triggered molecular signaling and metabolic pathway affecting Treg differentiation in mouse will be then thought in human colonic immune cells.

Ultimately, 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.

Title: **Metabolic control of IL-23 production by resident and migratory dendritic cells.**

Supervisor: **David DOMBROWICZ** – UMR 1011. Institut Pasteur de Lille. rue du Pr Calmette, 0320877967 - david.dombrowicz@pasteur-lille.fr

Background. Dendritic cells (DC) are key to the initiation of the adaptive immune response. They capture antigens in peripheral tissues and migrate to draining lymph nodes (LNs). In psoriasis (PSO), migrating IL-23-producing DCs activate T lymphocytes and IL-17 production in LNs. Metabolism controls key DC functions, and fatty acids exacerbate PSO through their effects on DCs, but the metabolic pathways governing these processes are poorly understood.

Aim. This project will investigate how DC metabolism and in particular the pentose phosphate (PPP) and hexosamine biosynthesis (HBP) pathways affect these parameters to induce psoriasis and contribute to their exacerbation in metabolic pathologies.

Methods. Preclinical in vivo and in vitro models, pharmacological inhibitors and gene modifications will be used to study a) the metabolic requirements of IL-23-producing DCs b) metabolic remodeling during exacerbation of psoriasis by a high-fat diet. The study will focus on enzymes controlling glycolysis and PPP respectively: Pfkfb3 and HBP: Gfpt1, Stt3a. Metabolic analyses based on flow cytometry (SCENITH) and transcriptomic analyses by scRNA-seq will be used.

Collaborations. This work will be undertaken in collaboration wtih Dr Stoyan Invanov (LP2M, Nice).

Keywords. DC, Psoriasis, Métabolism, scRNA-seq, bioinformatic, functionnal tests

Title : **Role of CX3CR1+ T lymphocytes in metabolic diseases**

Supervisors. **David DOMBROWICZ, Laurent L'HOMME** – UMR 1011. Institut Pasteur de Lille. rue du Pr Calmette. 59019 Lille. 0320877967 - david.dombrowicz@pasteur-lille.fr ; laurent.l-homme@inserm.fr

Context. CD4+ and CD8+ T lymphocytes are major players in adaptive immunity and play diverse roles in the development of metabolic diseases such as obesity, type 2 diabetes and metabolic fatty liver. In liver and adipose tissue, a T lymphocyte subpopulation expresses the CX3CR1 receptor, a receptor for the CX3CL1 chemokine involved in adhesion, migration, tissue retention and cell survival. The role of these CD4+ and CD8+ T lymphocyte subpopulations in the development of metabolic diseases is currently unknown.
Aim. In this project, a characterization and functional study of these subpopulations will be carried out.
Methods. The project will be based primarily on mouse models of obesity and steatosis/steatohepatitis (HFD, HFSCD and CDAA diets) and on flow cytometry. Other techniques such as cell culture, ELISA and RT-PCR as well as transcriptomic approaches (RNA-seq and scRNA-seq) will be used. Ultimately, the project should shed light on the role of these CD4+ and CD8+ T lymphocyte subpopulations in the development of metabolic diseases.
Keywords. T lymphocytes, metabolic diseases, MASLD, MASH, fractalkine.

Precision Health

Title: **PPARα and metabolic memory in diabetic retinopathy**

Tutors: **Anna Rita CANTELMO, David DOMBROWICZ.** U1011 - Récepteurs Nucléaires, Maladies Métaboliques et Cardiovasculaires, Institut Pasteur de Lille, Rue du Professeur Calmette, Lille - Tel: +33 320 87 71 48. anna-rita.cantelmo@univ-lille.fr

Diabetes mellitus affects an increasing global population, with diabetic retinopathy (DR) and diabetic macular edema (DME) being major causes of vision loss. Endothelial dysfunction plays a pivotal role in DR, where chronic hyperglycemia drives persistent metabolic and epigenetic alterations, contributing to 'metabolic memory' and disease progression.

Peroxisome Proliferator-Activated Receptor α (PPARα) serves as a key regulator at the intersection of metabolism and epigenetics. While primarily involved in lipid metabolism, PPARα also modulates inflammatory responses and influences DNA methylation. In diabetes, its expression is repressed due to hypermethylation, which may contribute to sustained endothelial dysfunction. Notably, PPARα agonists have demonstrated protective effects in DR, raising the question of whether their benefits stem from epigenetic modulation rather than metabolic regulation alone.

This project aims to: (1) assess endothelial PPARα epigenetic alterations as potential markers of vascular dysfunction in diabetes, and (2) investigate the interplay between metabolic and epigenetic pathways via PPARα in retinal endothelial cells. This study seeks to uncover novel endothelial targets for therapeutic intervention in DR.

Title: **Regulation of angiogenesis by mitochondrial protein import pathway.**

Tutor: **Anna Rita CANTELMO.** U1011 - Récepteurs Nucléaires, Maladies Métaboliques et Cardiovasculaires, Institut Pasteur de Lille, Rue du Prof Calmette, Lille - Tel: 03 20 33 70 78. anna-rita.cantelmo@univ-lille.fr

Mitochondria play a pivotal role in bioenergetics, metabolism, and apoptosis. Since the mitochondrial genome encodes only 13 proteins, the proper function of these organelles relies on the import of more than 1000 nucleus-encoded proteins. A crucial component of the mitochondrial protein import machinery is the evolutionarily conserved CHCHD4 oxidoreductase, which facilitates the oxidative folding of imported proteins after they after they cross the outer mitochondrial membrane. This finely regulated process is disrupted in disease conditions. This project employs a multidisciplinary approach, integrating molecular and cellular biology techniques, to:

i) Investigate the role and functional significance of CHCHD4 in endothelial cells;

ii) Characterize the signaling pathways influencing the CHCHD4-dependent import pathway in pathological angiogenesis.

The central hypothesis is that dysregulation of this import mechanism contributes to aberrant angiogenesis. Insights from this study may pave the way for novel therapeutic strategies targeting vascular dysfunction in cardiovascular diseases such as atherosclerosis.

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

Supervision: **Sophie LESTAVEL**. UMR 1011 INSERM - Laboratoire J&K, Pôle Recherche Faculté de Médecine, Bd 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.

Title: **Metabolic control of IL-23 production by resident and migratory dendritic cells.**

Supervisor: **David DOMBROWICZ** – UMR 1011. Institut Pasteur de Lille. rue du Pr Calmette, 0320877967 - david.dombrowicz@pasteur-lille.fr

Background. Dendritic cells (DC) are key to the initiation of the adaptive immune response. They capture antigens in peripheral tissues and migrate to draining lymph nodes (LNs). In psoriasis (PSO), migrating IL-23-producing DCs activate T lymphocytes and IL-17 production in LNs. Metabolism controls key DC functions, and fatty acids exacerbate PSO through their effects on DCs, but the metabolic pathways governing these processes are poorly understood.

Aim. This project will investigate how DC metabolism and in particular the pentose phosphate (PPP) and hexosamine biosynthesis (HBP) pathways affect these parameters to induce psoriasis and contribute to their exacerbation in metabolic pathologies.

Methods. Preclinical in vivo and in vitro models, pharmacological inhibitors and gene modifications will be used to study a) the metabolic requirements of IL-23-producing DCs b) metabolic remodeling during exacerbation of psoriasis by a high-fat diet. The study will focus on enzymes controlling glycolysis and PPP respectively: Pfkfb3 and HBP: Gfpt1, Stt3a. Metabolic analyses based on flow cytometry (SCENITH) and transcriptomic analyses by scRNA-seq will be used.

Collaborations. This work will be undertaken in collaboration wtih Dr Stoyan Invanov (LP2M, Nice).

Keywords. DC, Psoriasis, Métabolism, scRNA-seq, bioinformatic, functionnal tests

Title : **Role of CX3CR1+ T lymphocytes in metabolic diseases**

Supervisors. **David DOMBROWICZ, Laurent L'HOMME** – UMR 1011. Institut Pasteur de Lille. Rue du Pr Calmette. 59019 Lille. 0320877967 - david.dombrowicz@pasteur-lille.fr ; laurent.l-homme@inserm.fr

Context. CD4+ and CD8+ T lymphocytes are major players in adaptive immunity and play diverse roles in the development of metabolic diseases such as obesity, type 2 diabetes and metabolic fatty liver. In liver and adipose tissue, a T lymphocyte subpopulation expresses the CX3CR1 receptor, a receptor for the CX3CL1 chemokine involved in adhesion, migration, tissue retention and cell survival. The role of these CD4+ and CD8+ T lymphocyte subpopulations in the development of metabolic diseases is currently unknown.
Aim. In this project, a characterization and functional study of these subpopulations will be carried out.
Methods. The project will be based primarily on mouse models of obesity and steatosis/steatohepatitis (HFD, HFSCD and CDAA diets) and on flow cytometry. Other techniques such as cell culture, ELISA and RT-PCR as well as transcriptomic approaches (RNA-seq and scRNA-seq) will be used. Ultimately, the project should shed light on the role of these CD4+ and CD8+ T lymphocyte subpopulations in the development of metabolic diseases.
Keywords. T lymphocytes, metabolic diseases, MASLD, MASH, fractalkine.

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, 3ème étage OUEST. Faculté de Médecine de Lille, Pôle Recherche, 1, Place de Verdun, Lille. Phone number: 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 SRD5A1 enzymes playing critical roles. Interestingly, in obesity, a state of tissular cortisol overexposure contributing to the onset of metabolic complications has been described. 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. Previous studies using supraphysiological dose of GCs showed that SRD5A1 overexpression can rescue the impact of GCs on glucose stimulated insulin secretion (GSIS). As GCs have been shown to have a synergistic effect on β-cell dysfunction induced by a glucolipotoxic environment, the aim of the project would be to assess how SRD5A1 modulates the impact of physiological dose of GCs on GSIS in both human islets and INS1 cells cultured with medium enriched in glucose and lipids. All the experiments necessary for this project are mastered and routinely used in the lab.

Title: **Therapeutic Potential of SGLT4 Inhibition in Diet-Induced Metabolic Disease**

Supervisor: **Caroline BONNER**, Institut Pasteur de Lille / Inserm U1190 Translation Research of Diabetes - +33-(0)-32-06-23-413 - caroline.bonner@univ-lille.fr

The rising prevalence of obesity and type 2 diabetes (T2D) necessitates novel therapeutic approaches. We propose targeting sodium-glucose cotransporter 4 (SGLT4), which uniquely transports multiple sugars (mannose > glucose > fructose > 1,5-anhydroglucitol > galactose) via a Na+-dependent system with a Km of 2.6 mM. These sugars predominate in Western diets (WD) and exhibit elevated serum concentrations in individuals with obesity.

Our preliminary data with global Sglt4 knockout mice show remarkable protection against diet-induced obesity and T2D after five months of WD exposure. However, this model is limited as SGLT4 inhibition occurs from embryonic stages, whereas therapeutic intervention in humans would begin in adulthood after metabolic disease is established.

To address this critical question - can SGLT4 inhibition reverse already established metabolic disease? - we developed conditional Sglt4 knockout mice (Sglt4LoxP/LoxP). These mice will be crossed with tamoxifen-inducible UBC-CreERT2 mice to allow targeted SGLT4 deletion in adult mice after obesity is established. Crucially, all study groups will be maintained on WD for three months to develop obesity and metabolic dysfunction before inducing knockout, allowing us to determine if SGLT4 inhibition can drive weight loss and metabolic improvements in already obese mice. We will conduct comprehensive metabolic phenotyping, including body weight trajectories, glycosuria assessment, glucose homeostasis, and tissue-specific expression analyses of glucose transporters.

This project offers an opportunity to study a potential therapeutic strategy that could not only prevent but potentially reverse established metabolic disease.

Title: **Role of « ubiquitin-like protein » FAT10 in the hepatocyte suffering during MASH development**

Supervisor: **Réjane PAUMELLE-LESTRELIN**. INSERM-UMR 1011 "Nuclear receptor, metabolic and cardiovascular diseases". Laboratory J & K - Faculty of Medicine, Research Center - Bd Pr Jules Leclerc, Lille - Tel: 03 20 97 42 09 - rejane.lestrelin@univ-lille.fr

Metabolic dysfunction-associated steatotic liver disease (MASLD) affects 1/3 of the general population, which obesity is the main risk factor. MASLDs are characterized by an intrahepatic accumulation of lipids (steatosis) progressing to metabolic dysfunction-associated steatohepatitis (MASH) which can lead to the development of cirrhosis and hepatocellular carcinoma (HCC). To date, few effective medical treatments for MASH are available due to potential resistance to therapy. The disruption of degradation pathways leading to the formation of aggresomes: the Mallory-Denk-Bodies (MDB), and hepatocyte ballooning, the main histological characteristic of hepatocyte suffering, appears to be a potential mediator of the progression of MASH to cirrhosis and HCC. However, molecular mechanisms contributing to their formation are not known. Our transcriptomic analysis of liver biopsies from obese patients with MASH shows that FAT10 expression correlates positively with different histological grades of MASLD and MASH severity such as MDB. Interestingly, FAT10 is a protein of the "ubiquitin-like" family involved in FATylation processes regulating protein degradation, and is induced by inflammation in metabolic tissues. Interestingly, FAT10 plays a role in the formation of MDB induced by the hepatotoxic agent (DDC) in mice, suggesting that FAT10 may play a role in MDB formation during MASH progression. Our project therefore aims to characterize the role of FAT10 in the formation of MDB and hepatocyte suffering during the MASH severity in murine et human cellular models. This project will identify cellular and molecular mechanisms contributing to the hepatocyte suffering associated to MASH development and progression to cirrhosis and may lead to the identification of new therapeutic targets.

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

Supervisors: **Réjane PAUMELLE-LESTRELIN** and **Guillaume LASSAILLY** (U1286). INSERM-UMR1011 "Nuclear receptor, metabolic and cardiovascular diseases". Laboratory J&K - Faculty of Medicine, Research Center-Bd Pr Jules Leclerc, Lille. Tel: 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 clinical relevance of FAT10 expression in hepatocytes associated with type 2 diabetes status in liver biopsies from obese patients with or without MASH.

Title: **Identification of FAT10 interaction partners involved in hepatocyte injury through a proteomic approach**

Supervisor: **Audrey HELLEBOID**. INSERM-UMR1011 "Nuclear receptor, metabolic and cardiovascular diseases". Laboratory J & K - Faculty of Medicine, Research Center - Bd Pr Jules Leclerc, Lille - audrey.helleboid@univ-lille.fr

Metabolic dysfunction-associated steatotic liver disease (MASLD) affects about one-third of the general population, with obesity being the primary risk factor. This condition is characterized by the accumulation of lipids in the liver (steatosis), which can progress to metabolic dysfunction-associated steatohepatitis (MASH). This condition can lead to the development of cirrhosis and hepatocellular carcinoma (HCC). To date, there are few effective pharmacological treatments for MASH, likely due to resistance mechanisms. Disruptions in protein degradation pathways, leading to the formation of Mallory-Denk bodies (MDB) and hepatocyte ballooning, typical markers of liver damage, may play a key role in the progression of MASH to cirrhosis and HCC. Our transcriptomic analyses of liver biopsies from obese patients with MASH reveal that FAT10 expression is positively correlated with MASLD severity. FAT10, a protein belonging to the "ubiquitin-like" family, is involved in FATylation, a process regulating protein degradation, and is induced by inflammation in metabolic tissues. Studies in mice show that FAT10 is involved in the formation of MDB induced by the hepatotoxin DDC, suggesting it may also play a similar role in the progression of MASH. Our project aims to characterize the role of FAT10 in hepatocyte injury during the progression of MASH. Identifying its interaction partners through a proteomic approach will help characterize the molecular pathways involved in liver damage and understand the role of FATylation in this process, with the hope of identifying new therapeutic targets.

Title: **Role of the nuclear receptor Rev-erbα in angiogenesis**

Supervisor: **Benoit POURCET** – Université de Lille, INSERM U1011 Institut Pasteur de Lille CHU Lille EGID – 01 rue du Pr Calmette – 0320877125 - benoit.pourcet@univ-lille.fr

Atherosclerosis is a chronic inflammatory disease of large vessels triggered by the accumulation of cholesterol and leukocytes in the vascular wall. During atherogenesis, vascular wall thickening induces local hypoxia and promotes the vasa vasorum expansion by angiogenesis. These neovessels are however immature and then promote leakage of lipids and leukocytes thus contributing to plaque progression and rupture. The molecular and cellular mechanisms involved in the growth of the perivascular blood network are not known. Reducing its expansion could, however, represent an innovative therapeutic strategy in the treatment of these diseases. Our preliminary data suggest that the nuclear receptor Rev-erb-α controls angiogenesis and intraplaque neovascularization ex vivo and in vivo. This proposal aims to determine the impact of Rev-erb-α in endothelial cells during angiogenesis using in vivo and in vitro approaches. For that purpose, angiogenesis will be assessed in vivo by confocal and light sheet microscopy in endothelial-specific Rev-erbα-/- mice and their control by analyzing the development of the vascular network of newborn retinas. The role of Rev-erb-α on angiogenic processes will then be analyzed in vitro using 3D spheroid models of cell competition. The pathways involved in angiogenesis will be assessed in tissues and cultured cells by WES and RT-qPCR. This M2R proposal aims to determine the impact of Rev-erb-α in angiogenesis during atherosclerosis and to define the molecular and cellular mechanisms involved.

Title: **Evaluation of pharmacological therapies for MASH, liver fibrosis and atherosclerosis in a new preclinical mouse model combining MASLD and atherosclerosis development**

Supervisor: **Fanny LALLOYER**, Inserm UMR 1011 - Institut Pasteur of Lille - University of Lille. Tel : +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 and in conjunction with cardiometabolic risk factors. 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. However, the majority ofMASLD patients die from cardiovascular diseases. Currently, there is only one pharmacological treatment, resmetirom, approved in the USA for MASH, the aggressive form of MASLD.

In the laboratory, we have set up a new mouse model which progressively develops all stages of human MASLD pathology (liver steatosis, inflammation, ballooning and fibrosis) under high fat diet in a short period of time. The project aims to better understand MASH and liver fibrosis physiopathology and to test novel therapeutic targets for MASLD and its consequences on atherosclerosis development in this model. Histological, biochemical and molecular analyzes will be carried out on the various technical platforms of the laboratory.

Title: **Role of RAGE Antagonists in the Control of Aging**

Supervisor: **Chantal FRADIN**, U1167 RID-AGE, BioPrev: from inflammaging to prevention. Tel: 0320623486 - chantal.fradin@univ-lille.fr

Ageing is associated with the appearance of low-grade systemic and sterile inflammation, known as inflammaging, which has a significant impact on the quality of ageing and the onset of age-related diseases. RAGE (receptor for advanced glycation end products) is a multi-ligand receptor that appears to play an important role in the induction and maintenance of this inflammation. Blocking RAGE-induced inflammatory signalling may limit inflammaging and its deleterious effects on ageing. To verify this hypothesis, new RAGE antagonists with improved affinity and inhibitory potential have been synthesised. The aim of the project is to analyse the anti-ageing potential of these molecules in one of the key animal models in the biology of ageing, the nematode Caenorhabditis elegans. Several transgenic strains expressing human RAGE in different tissues (intestinal, neuronal and muscle) are available to test whether RAGE antagonists can control the appearance of hallmarks of ageing and improve longevity. Endogenous and/or exogenous ligands such as AGEs and the S100A6 protein will be used to activate RAGE. Different physiological tests (measuring the mobility and motility of the worms) and molecular tests (analysing the integrity of muscle fibres, the antioxidant response, innate immunity and protein homeostasis) will be performed to analyse the effect of RAGE antagonists on ageing.

Title: **Isolation of the Man2GlcNAc2 epitope from neuroblast differentiation-associated protein, AHNAK, to decipher its molecular recognition by antibody and lectin probes for future diagnostics**

Tutor: **Julie BOUCKAERT**, UGSF, UMR 8576 CNRS / Université de Lille +33(0)362531729, julie.bouckaert@univ-lille.fr

AHNAK, or neuroblast differentiation-associated protein, is a huge scaffolding protein (629 kDa) involved in various physiological processes, including calcium homeostasis and cellular signaling. AHNAK was initially identified as desmoyokin, a component of desmosome plaques in stratified squamous epithelial cells. It plays a role in muscle contraction, kidney development, and tumour biology. AHNAK has an essential role is neurogenesis but it was also found involved in brain cancer. We found that AHNAK carries a paucimannose N-glycan structure, Man2GlcNAc2, consisting of two mannose and two N-acetylglucosamine residues. The presence of this glycan structure is intriguing because it has recently been acknowledged as an important HLA-II immunopeptide. Its Man2GlcNAc2 glycosylation may thus be a crucial regulator of AHNAK's scaffolding function in neuroblast differentiation. From our interests, the Man2GlcNAc2 epitope could serve as a unique molecular target for diagnostic purposes in cancer immunobiology. The objective of the master2 thesis is to evaluate molecular probes against the Man2GlcNAc2 epitope. This includes the recombinant protein production of an AHNAK fragment, followed by isolation and enrichment of the particular glycopeptide using lectin affinity chromatography. Molecular interaction techniques will assist to select the better molecular probe among already in-house glycan-specific antibodies, lectins and nanobodies.

Keywords:
Man2GlcNAc2, paucimannose, AHNAK, Mannitou, lectin, nanobody

Title : **Differentiation of genome-edited induced pluripotent stem cells (cellular models of MLH1 constitutional epimutations) into colonic organoids**

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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. To explore these molecular mechanisms, we created cellular models of MLH1 constitutional epimutations based on induced pluripotent stem cells (iPSC). We used the CRISPR-Cas9 technology to modify the MLH1 gene and introduce genetic variants identified in epimutation carriers and associated in cis with promoter hypermethylation (secondary epimutations). While these stem cells showed de novo MLH1 methylation with increasing levels, methylation should be stable in differentiated cells.

The aim of the project is to differentiate these genome-edited iPSC into colonic organoids. This will be done in the ORGALille platform (https://orgalille.univ-lille.fr/) where the student will be trained to iPSC culture and organoid differentiation. Further characterization of the organoids will involve (1) study of their methylation profile using dedicated technics (2) immunofluorescent labeling (3) mutational and microsatellite instability analyses.

Key words: Epigenetics; Oncogenetics; induced pluripotent stem cells; organoids; methylation.