Abstracts of oral communications

KIF20A-mediated endosomal sorting: molecular mechanism and impact in cancer progression

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Emerging evidence shows that multiple endocytic proteins are dysregulated in cancer, leading to alteration in membrane trafficking. The spatial organization of endocytic trafficking requires motor proteins that transport organelles and cargoes along actin and microtubules cytoskeletons. Together, they also generate force for membrane remodelling. Kinesin family member 20A (KIF20A), a plus-end microtubule motor, is known to mediate spindle formation during late mitosis. During interphase, it participates in the fission process of Rab6-positive vesicles from the Golgi complex through its interaction with MyosinIIA. KIF20A is upregulated in many cancers, a feature that correlates with tumor progression, tumor aggressiveness and poor overall survival. Recent studies underline a new role of KIF20A in cell adhesion and migration. In fact, KIF20A inhibition was shown to affect cell proliferation, but also cancer cell motility and invasion.

Our project aims to determine the molecular mechanism by which KIF20A and its partners participate in tumor cell motility by regulating efficient membrane recycling of key motility proteins. Studying the role of KIF20A in the intracellular trafficking of Integrin β1 in cancer cell lines, we highlight a new function for this kinesin in the endocytic pathway. We present evidence that, in several cell lines, KIF20A motor activity inhibition disrupts the homeostasis of the endosome, as evidenced by the enlargement of early and late endosomes. In KIF20A inhibited conditions, a delay of transferrin receptor recycling was observed, and it was instead directed to the enlarged endolysosomal compartment. Furthermore, an accumulation of branched actin accompanied by abnormaly long tubules is observed around enlarged endosomes. We propose that KIF20A is a microtubule-dependant motor that influences actin dynamics on endosomes and thus endosomal sorting, and that its overexpression could promotes highly dynamic trafficking of key metastatic phenotype proteins.

Keywords: Trafic, endosomes, actin, cancer, metastasis

Deciphering the Myeloid Landscape by Combining A Single-Cell Transcriptomic Analysis with Imaging in NSCLC Patients

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Tumor-associated macrophages (TAMs) represent a significant proportion of the infiltrating leukocytes and are often associated with unfavorable prognoses due to their involvement in tumor growth, and immune suppression. However, lung resident macrophages exhibit heterogeneity of origin, function, and anatomic distribution (stroma, tumor nodules, or tertiary lymphoid structures (TLS)). We hypothesized that the composition of the myeloid compartment shapes the local adaptive immune response.

We aim to characterize different “myelotypes “in NSCLC and the molecular pathways of interactions between myeloid cells and T- and B-lymphocytes. We expect to identify the molecular mechanisms involved in the regulation (activation or suppression) of the immune response mediated by the myeloid compartment.

Using phenotypic mass cytometry analysis, we identified tumor-associated monocytes (TAMo) CCR2+ CD36+, and distinct TAM subsets based on CD169, and CD206 expression. Multiplex fluorescent microscopy unveiled that CD169+ CD206+ TAM mostly represented alveolar macrophages (AM). Using a public scRNAseq dataset of CD45+ cells of NSCLC, we recovered the transcriptomic signatures of TAMo and AM; and we further defined 4 populations among interstitial macrophages (IM) according to the expression of the canonical markers: SPP1, FOLR2, IL4I1, and LYVE1. The relative proportions of these myeloid subsets were used to define the global myeloid landscape in each individual. Hierarchical clustering based on these myeloid landscapes highlighted 3 main “myelotypes”: A- “IM/AM-rich”; B- “IM/AM-poor”, and C- “myeloid-poor”. Further association of these myelotypes with B- and T-cell transcriptomic profiles unveiled that myelotype “B” was associated with significant infiltration of plasma cells whereas myelotype “A”, was associated with a lower cytotoxic signature CD8+ T-lymphocytes signature.

Our findings suggest close associations between the myeloid landscape and the adaptive response. Further, interactome studies will help us to identify potential molecular pathways involved between TAMs and plasma cells or CD8+ T cells, to define potent immunomodulators of these axes for therapeutic approach.

Keywords: NSCLC, interactome, macrophage, monocyte, immune archetypes

CYYR1 is a novel regulator of the E3 ubiquitin ligase WWP1 with favorable prognosis value in breast cancer

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Ubiquitination plays a crucial role in cellular homeostasis by regulating the degradation, localization, and activity of proteins, ensuring proper cell function and balance. Among E3 ubiquitin ligases, WWP1 is implicated in cell proliferation, survival and apoptosis. Notably WWP1 is frequently amplified in breast cancer and associated with poor prognosis. Here we identify the protein CYYR1 that had previously no assigned function, as a regulator of WWP1 activity and stability. We show that CYYR1 binds to the WW domains of the E3 ubiquitin ligase WWP1 through its PPxY motifs. This interaction triggers K63-linked auto-ubiquitination and subsequent degradation of WWP1. We further demonstrate that CYYR1 localizes to late endosomes and directs poly-ubiquitinated WWP1 toward lysosomal degradation through binding to ANKRD13A. Moreover, we found that CYYR1 expression attenuates breast cancer cell growth in anchorage-dependent and -independent colony formation assays in a PPxY-dependent manner. Finally, we highlight that CYYR1 expression is significantly decreased in breast cancer and is associated with beneficial clinical outcome. Taken together our study suggests tumor suppressor properties for CYYR1 through regulation of WWP1 auto-ubiquitination and lysosomal degradation.

Keywords:CYYR1, WWP1, ubiquitination, breast cancer

Alterations of the DNA Damage Response in MSI Cancers and Their Contribution to Tumorigenesis and Therapeutic Response

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Microsatellites Instability (MSI) is one of the main types of genome instability, observed in roughly 4% of cancers. It is found in about thirty primary locations, with the highest frequencies observed in endometrium, colon and stomach. It is caused by the functional inactivation of the mismatch repair system (dMMR), which drives an hypermutator phenotype characterized by the accumulation of indel mutations at microsatellite DNA repeats (mutational hotspots).

Many key genes involved in the DNA damage response (DDR) are recurrent targets of MSI in these tumors and we hypothesize a major physiopathological and clinical impact of some of these mutations, which remains largely unexplored. The multiple somatic alterations targeting these key processes also suggest that dMMR cells rely on specific adaptive mechanisms which may constitute clinically-targetable vulnerabilities. This project aims to delineate the landscape of DDR gene alterations and associated pathways in MSI tumors. We seek to identify events with pro-/anti-oncogenic potential, along with predictive signatures of patient outcomes and responses to various treatments, including chemotherapy and immunotherapy. To achieve this, we leverage multi-omic and clinical data sourced from a large collection of MSI samples curated by our team.

During my first year I designed a comprehensive database aggregating whole exome sequencing and clinical data for over a thousand samples. This database streamlines data analysis, extraction, and integration processes. To identify patterns of positive/negative selection within DDR MS variants, we used a previously established model of MS instability based on MS length and mutation frequency. I‘ve developed new tools specifically designed for computing tumor purity and variant allele frequency in MSI tumors. A new model based on this new data is underway, some result are available and we’re still fine-tuning the precision of the model. We will present the preliminary results of this approach and discuss the perspectives of the project.

Keywords: Bio-informatics, Oncology, Database, Modelisation

Promising CD44 molecularly imprinted nanoparticles for targeting breast cancer stem cells

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Triple-negative breast cancers are characterized by the highest content of breast cancer stem cells (BCSCs), giving them a very-aggressive and drug-resistant form of malignancy. Although antibody-drug conjugates (ADCs) can work efficiently against these cancers, instability and high production costs are some of their drawbacks in clinical use.
The main focus of this project is to design molecularly imprinted polymers (MIPs) for the targeting of cluster of differenciation 44 (CD44), a transmembrane receptor over-expressed in BCSCs and, hence, more-specific anticancer drug delivery. This approach can provide a potential alternative to ADCs in therapy applications.

The development of MIPs anti-CD44 (MIPs-CD44) was performed by a novel double layer imprinting approach based on the solid-phase method. This strategy leads to the preparation of MIPs, which were combined with SN-38 drug and targeted toward a linear characteristic peptide of CD44. SN-38 is an active metabolite of the topoisomerase I inhibitor, irinotecan, that inhibits DNA and RNA synthesis. The size and the morphology of nanoparticles were characterized by transmission electron microscopy and they exhibited a diameter of 200 nm, compatible with biological applications.

To evaluate the binding efficiency of MIPs-CD44 to their target region, two breast cancer cell lines, SUM-159 and MCF-7, were incubated with fluorescent MIPs-CD44 and analyzed by flow cytometry. According to the results, SUM-159, which express the highest amount of CD44, show the highest binding of MIPs. Conversely, MCF-7 cells, which express low amount of CD44, did not show appreciable binding by MIPs. This specific binding was confirmed by confocal microscopy in 2D and 3D cell models. Moreover, evaluation of cytotoxicity demonstrates the biocompatibility of MIPs and the encapsulation efficiency of SN-38 molecule into the nanoparticles.

These MIPs-CD44 is a promising nanocarrier capable of specifically targeting and controlling drug delivery to BCSCs, and can provide new therapeutic treatments against clinically relevant targets.

Keywords: Molecularly imprinted polymers (MIPs), breast cancer stem cells (BCSCs), CD44 proteins, targeted therapy, nanomedecine

Established new immunodeficient large animal model for receiving human induced pluripotent stem cell-derived hematopoietic grafts.

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CRSA Delhommeau

Hematopoietic cell transplantation (HCT) is the first lifelong cell therapy treatment available for many blood disorders, including leukemia, sickle cell disease and autoimmune diseases. The demand for clinical-grade HSCs has increased significantly in recent decades, leading to major difficulties in treating patients.
To fulfill this need, we propose large-scale production of hematopoietic stem cells from human induced pluripotent stem cells (hiPSC) in an Advanced Therapy Medicinal Production (ATMP) core facility. We also plan to establish a new large animal model for human HCT.

We developed a robust, transgene-free, protocol based on the differentiation of human induced Pluripotent Stem Cells into bona fide transplantable human HSC. This procedure is based on the differentiation of embryoid bodies with morphogens and cytokines to generate HSC over a 17-day culture period.
For the technology transfer and scale-up of our protocol, we worked closely with Atlantic Bio's GMP (ABG) ATMP Core Facility. The large-scale produced HSCs were subjected to in vitro and ex vivo quality controls, including cytometric validation against predefined markers and transplantation into irradiated NSG mice.
In parallel, we decided to work with the Aachen mini-pig model. A 6-week-old Aachen pig weighs about 6 kg, which is compatible with pediatric oncology cases. We developed an Aachen-specific conditioning regimen for human HCT, i.e. the radiation dose for aplastic anemia and the different immunosuppressive drug combinations. The mini-pigs were monitored and blood samples were taken and analyzed at different time points. We had to take into account the innate immunity of the Aachen mini-pigs and find drugs to temporarily switch it off.

We successfully scaled-up HSC production with the ABG. The produced cells were compared to their princeps counterparts by FACS analysis, and subsequently injected into mice where they successfully recapitulated full human hematopoiesis.
We set the Aachen conditioning regimens. Innate immunity was a challenge, but injection of Cobra Venum Factor showed a convincing protective effect of human cells by inhibiting porcine complement.

Now that the HSC production scale-up and mini-pig conditioning protocols are in place, the HCT large animal study can now begin. This proof of concept in a large animal will be a major step towards hiPSC-derived transplants and thus improved access to treatment for patients.
Keywords: Hematopoietic Stem Cells - Grafts – hiPS – large animal model

Development of innovative massively multiplexed serological tests profiling humoral responses to infections or vaccinations

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The management of COVID-19 pandemic at local, regional, national and international levels demonstrated the value of epidemiological monitoring of serology across territories, in order to better adjust and anticipate preventive and curative health measures. Drawing on this experience, the French government is studying the possibility of profiling the humoral immunity of individuals at different age groups (ages 25, 45 and 65) for their immunity conferred by compulsory and recommended vaccinations. In France, this would represent around 2.5 million tests per year. The aim and benefits are to inform individuals of the booster doses they need to be fully protected, to help regions target needs and adjust logistics, and to help nations update vaccination policies and protocols. To carry out such surveillance and integrate them into a reimbursement protocol, a rapid, multiplexed in vitro test technology will be needed to reduce costs and adapt it to very high throughput on existing commercial equipment for analytical laboratories.

The aim of my thesis project is to develop the use of fluorescent bar-coded mineral microbeads, developed by the Paris-based company Nexdot, to massively multiplex serological immunoassays. We began by developing fluorescent probes for the detection of human immunoglobulins. Then, in collaboration with Nexdot's team of chemists and engineers, we developed microbeads and optimized their surface chemistry to be able to anchor target proteins to the surface of these objects. These developments are now enabling us to adapt our technology to patient blood samples. The implementation of this technology will enrich epidemiological studies by mapping vaccination status and past infections in the French population.

Keywords: Diagnosis, serology, vaccinology, epidemiology

Study of human thymic macrophages in Myasthenia Gravis

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Myasthenia Gravis (MG) is a rare autoimmune disease primarily caused by autoantibodies targeting the acetylcholine receptor (AChR) at the neuromuscular junction. In AChR-MG, the thymus is the effector organ. It is characterized by a strong inflammatory signature and ectopic germinal center development orchestrated by the production of interferon (IFN)-ß. The release of IFN-ß might result from the presence of endogenous nucleic acids of improperly processed apoptotic thymocytes. We have recently shown that macrophages are significantly decreased in the thymus of AChR-MG patients.

To date, human thymic macrophages have not been thoroughly studied. This study aims to investigate the link between macrophages and thymic inflammation in AChR-MG. Therefore, we established and optimized single-cell RNA sequencing (SC-RNAseq) and analyzed 2 thymuses from adult donors and 3 thymuses from AChR-MG patients. Imaging mass cytometry (IMC) experiments were performed on 1 adult thymus and 1 AChR-MG thymus to analyze macrophage subpopulations. Our results reveal a more inflammatory transcriptome of macrophages in AChR-MG patients compared to control by SC-RNAseq, while IMC show several macrophage populations with distinct localization and phenotype and indicate a potential role of this cells in the origin or the maintenance of germinal centers in AChR-MG thymus.

Keywords: Myasthenia Gravis, Macrophage, Thymus, Inflammation, Autoimmune disease

Poor FcRn recycling accounts for the limited increase of Fc-fused Factor VIII half-life

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Fc-fused FVIII (rFVIIIFc) is used to correct bleeding in hemophilia A patients. The 1.5-fold increase in half-life achieved upon fusing FVIII to the Fc fragment is disappointing as compared to the gain obtained for rFIXFc (3-fold). Such a limited increase in half-life is explained by the binding of the FVIII moiety to its chaperon, von Willebrand factor (VWF), which favors the catabolism of the FVIII/VWF complex by VWF-specific receptors. The recycling of Fc-fused proteins involves the binding, following endocytosis, of their Fc fragment to the neonatal Fc receptor (FcRn) at acidic pH. The protein is thus rescued from lysosomal degradation, recycled at the cell surface and release from FcRn at neutral pH. Interestingly, positive charges in the variable regions of human monoclonal IgG were shown to affect the recycling of the IgG by favoring interaction with the FcRn at neutral pH, thus preventing extracellular release and fostering lysosomal degradation. Because the FVIII C1 and C2 domains contain positively charged residues, we hypothesized that, in addition to endogenous VWF mediated catabolism, binding of FVIII to the FcRn may play a role in its poor recycling and hence short half-life.

Binding to the human (h) and mouse (m) FcRn was studied by surface plasmon resonance at pH6 and 7.4 using a Biacore and ELISA. Electrostatic potential of molecules were calculated using the Coulomb method. Modeling of the rFVIIIFc mutant was performed using I-TASSER. Half-lives were evaluated in VWF and FVIIIKO mice.

At pH6, the binding affinities of rFVIIIFc, rFIXFc and the two IgGs m66.6 and VRC01 were similar both for mFcRn and for hFcRn. At pH7.4, rFVIIIFc and m66.6 -positevely charged in the variable region- bound both mFcRn and hFcRn, while rFIXFc and VRC01 did not. Ion strength ELISA demostrated the implication of positively charged residues in the binding of rFVIIIFc and m66.6 to FcRn at neutral pH. Masking the C1 or C2 domains of FVIII using F(ab')2 fragments from specific monoclonal IgG reduced the binding of rFVIIIFc to FcRn at neutral pH. Anti-A2 F(ab')2 fragments had no effect. A rFVIIIC1C2Fc mutant, with reduced positive electrostatic potental in the C1 and C2 domains bound normally to FcRn at pH6 and marginally at pH7.4.Accordingly, FVIIIC1C2Fc showed a longer half-life than rFVIIIFc in VWF-KO mice, but not in FVIII-KO mice.

Binding to FcRn of the C1 and C2 domains of Fc-fused FVIII at neutral pH favours rFVIIIFc routing towards lysosomes: mutations in C1 and C2 prevented rFVIIIFc binding to FcRn at neutral pH, decreased its intracellular degradation and increased its half-life in vivo. We hypothesize that binding to endogenous VWF is not the only reason for a poor gain in half-life of therapeutic rFVIIIFc.

Keywords: Hemophilia A, Factor VIII, FcRn-recycling, half-life

How an acute stress impacts immunity in elderly patients?

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Our society faces a major challenge with the management of the health and socio-economic burden caused by aging of the population (older than 75 years). As society ages, the incidence of physical limitations is dramatically increasing, which reduces the quality of life and increases healthcare expenditures. In western society, ~20% of the population over 60 years is confronted with moderate or severe physical limitations. This fragility results in a higher morbidity and mortality where the deleterious role of inflammation is often debated. In this context, we used hip fracture (HF) as an acute stress model that accelerates the progressive course of aging. Nowadays, this trauma, which affects around 1.6 M patients worldwide, is still associated with poor clinical outcomes in the elderly (20-30% one-year mortality; 50% inability to walk). This emphasizes the value of assessing biological factors that may predict clinical outcome after HF.

Our aim is to decipher mechanisms taking place during this medical situation, by comparing immunity from patients with different clinical outcomes (autonomy or death) in order to decrypt the respective pathways involved.

We analyze longitudinally immunological parameters evocating of the Immune Risk Phenotype in sequential pre- and post-surgical samples collected from HF patients over 75 years of age. Clinical outcomes (death and capacity to walk) were collected retrospectively. The different markers, such as white blood cells count, circulating T- B- & NK-cells (naïve/ memory/activation status), CMV responsiveness, and inflammatory molecules were screened by flow cytometry and Luminex to determine the immune status of such patients.

Results: The study revealed that HF is associated with a profound impairment of immunity. Comparing healthy elderly individuals and HF elderly patients, we found a transient T-cell leucopenia and an acute hyper-inflammation early post fracture. Among this signature, we pinpoint a central role of neopterin (an immune activation marker) which predicts the loss of autonomy and death.

Both innate and adaptive immunity are affected transitory during this medical event which leads to different immune trajectories. The identification of these pathways could result in the development of new therapeutic strategies for better care of the geriatric population.

Keywords: Inflammation, Immune regulation, Immune dysfunction, Senescence

Autocrine function for hepcidin in Kupffer Cells during iron overloading.

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Iron metabolism is tightly regulated so iron is available for biological functions while preventing its cytotoxic effects. Macrophages play a central role in establishing this balance. Kupffer Cells (KCs), liver resident macrophages, are involved in the elimination of damaged erythrocytes, ensuring systemic iron recycling to prevent damage from excess iron deposition in organs. Moreover, the hepcidin/ferroportin axis is critical to coordinate cellular iron export. Hepcidin induces the degradation of the iron exporter ferroportin, suppressing iron release from macrophages and parenchymal cells. The hepatocyte-derived hepcidin control on systemic iron homeostasis is well-known, but the autocrine role of hepcidin produced by the macrophage remains to be more defined.

Here, we observed that iron overloading by iron dextran injection into wild-type mice resulted in a rapid and partial loss of embryonically-derived KCs (EmKCs composing the KC pool in healthy livers). Concomitantly, monocyte-derived macrophages were recruited to the liver and acquired the prototypical KC marker CLEC2. However, engraftment of these monocyte-derived KCs was only transient, and EmKCs proliferated to replenish the KC pool later. Similar studies of iron overloading were replicated in mice with hepcidin deficiency in macrophages (Lysm-Cre x Hamplox/lox), including KCs. Decreased EmKCs density was rapidly observed in both Lysm-Cre x Hamplox/lox and Hamplox/lox control mice following iron dextran injection. However, EmKCs in transgenic mice exhibited both a reduced proliferation rate and a diminished pool as compared to control mice.

These preliminary observations support the contention that hepcidin, in an autocrine way, plays a role in Kupffer Cells' response to iron overloading.

Keywords: iron, liver, macrophage, hepcidin

Role of adipose tissue macrophages in β3 adrenergic-mediated beige adipogenesis and thermogenesis.

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Obesity results from an imbalance between calories ingested and expended. The adipose tissue plays a central role in obesity development as it coordinately regulates lipid storage and utilization. White adipose tissue (WAT) depots are primarily specialized in the storage of lipids while the brown adipose tissue (BAT) has the capacity to dissipate energy in the form of heat through the mitochondrial uncoupling protein 1 (UCP1). Pharmacological stimulation of B3-adrenergic receptors enhances BAT UCP-1 expression and favours the generation of UCP1+ adipocytes in the WAT, a process called beige adipogenesis. Therefore, unlocking the thermogenic potential of beige adipocytes to regulate whole-body energy homeostasis could be a therapeutic approach to limit obesity and the metabolic syndrome. B3-adrenergic stimulation is accompanied by a massive infiltration of immune cells in the WAT, whose contribution to beige adipogenesis remains elusive. Here, we set out to study whether inflammatory cells participate to beige adipogenesis.

In response to B3-adrenergic stimulation (CL-316,243, 1mg/kg for 3 days), we observed marked accretion of macrophages originating from circulating monocytes (moMacs) in the WAT and BAT. Such accumulation was particularly striking in the visceral WAT (vWAT) . We then found that CL-induced WAT lipolysis was essential to drive moMacs accumulation. Moreover, we noticed that monocyte mobilization to the WAT was induced after the first CL injection, while subsequent injections did not further induce monocyte recruitment. Eventually, moMacs were critical to mediate beige adipogenesis in the vWAT as evidenced by reduced Ucp1 mRNA induction in CL-treated Ccr2-/- mice that exhibit very low levels of circulating monocytes and moMacs accumulation.

Our preliminary results outline an interrelated network of physiological events including lipolysis and inflammation, that coordinates beige adipogenesis in the vWAT and hence prompt us to further explore the role of moMacs in this intricate beiging mechanism.

Keywords: B-adrenergic stimulation, Inflammation, Macrophages, beige adipogenesis, Obesity

Untying the role of Kupffer cells in the pathophysiology of acute liver failure ?

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Acute Liver Failure (ALF) results from hepatocyte death exceeding liver regeneration capacities. Chronic liver condition, including liver metabolic diseases, represent a major risk factor for hepatic decompensation, then termed acute on chronic liver failure (ACLF). Moreover, patients with chronic liver conditions are prone to bacterial infections, a key triggering event of ACLF. ALF/ACLF pathophysiology is characterized by cytokine-induced hepatocyte cell death, suggesting Kupffer cells (KCs), liver-resident macrophages, could play an important part given their pathogen recognition and cytokine production abilities. We thus aim to untie their role in ALF/ACLF pathophysiology.

We induced ALF/ACLF in mice using lipopolysaccharides (LPS) injection with D-galactosamine or chronic liver conditions (steatohepatitis or cholangiopathy) to sensitize hepatocytes to cytokine-induced cell death. Mice with KC-specific deletion for Tlr4 (Tl4ΔKC), Il10 (Il10ΔKC) or Bhlhe40 (Bhlhe40ΔKC) were used to respectively study LPS-induced KCs activation, the impact of KCs' secretion of IL-10 anti-inflammatory cytokine, and Bhlhe40's role in balancing cytokine production during ALF/ACLF pathophysiology. We demonstrated that Tl4ΔKC mice exhibited reduced TNFα levels, leading to decreased liver damages and improved survival post-LPS injection. Additionally, LPS stimulated IL-10 production by KCs in those mice. We then observed increased susceptibility to hepatocyte death and liver damages in Il10ΔKC mice, highlighting IL-10 hepatoprotective function in ALF/ACLF. Moreover, we identified Bhlhe40 as a transcription factor possibly regulating Tnf expression. As expected, Bhlhe40ΔKC mice showed decreased TNFα levels and were protected from ALF/ACLF severity.

Our findings establish the dual role of KCs in ALF/ACLF pathophysiology, by simultaneously promoting the production of pro and anti-inflammatory cytokines involved in hepatocyte cell-death. We identified Bhlhe40 transcription factor as a potential regulator of ALF/ACLF and highlighted the predominant contribution of KC-specific production of TNFα in the course this condition.

Keywords: CD36, Cancer, metabolism, ferroptosis

Oxidative stress influences the evolution of a reductionnist community of gut bacteria and bacteriophage.

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Reactive oxygen species (ROS) play a central role in the pathogenesis of inflammatory bowel diseases (IBD). These diseases are characterized by an altered intestinal microbiota. We hypothesised that ROS would affect the diversity of bacteria and their bacteriophages (phages) and designed a study aimed at identifying the impact of ROS-induced oxidative stress on the interactions between them.

We set up a reductionist in vitro model using a defined microbial community of three Escherichia coli strains and a virulent phage during continuous culture in chemostats. We mimicked oxidative stress by adding hydrogen peroxide and compared the evolution of this community by studying population dynamics and the profiles of bacterial resistance and phage host-range over time, in the presence or absence of stress.

Phage and bacterial populations co-existed over 10 days in both conditions. However, hydrogen peroxide impacted phages-to-bacteria ratios and the evolution of the phage host range. Indeed, phage populations decreased in concentration and evolved to be more specialist under oxidative stress compared to the control condition. In bacteria, we observed an increase in resistance to infection in the presence of oxidative stress. Finally, time-shift experiments coupled with genomic analysis highlight potential signatures of phage-bacteria arms race.

These data contribute to the fundamental understanding of how environmental variations may affect bacteria-phage interactions and hence the equilibrium of the gut microbiota. Determining the impact of inflammation-driven abiotic factors in altering microbial diversity is a step towards understanding the pathophysiology of IBD.

Keywords: bacteria; bacteriophage; coevolution; oxidative stress

Intermediate filament synemin acts as a regulator of cell remodelling during cardiac hypertrophy

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Synemin, an intermediate filament expressed in skeletal, cardiac and smooth muscle, is associated with the protein kinase A (PKA) through its tail domain, acting as an A-kinase-anchoring protein (AKAP) that regulates the spatial and temporal targeting of PKA activity. Previously we found that mechanical overload of skeletal muscle induces fiber hypertrophy in synemin-knockout mice. Moreover, two missense mutations near the AKAP domain have been identified in human patients with dilated cardiomyopathy.

Our aim is to investigate the impact of synemin in cardiac hypertrophy by a combination of in vivo and in vitro models. Functional, morphological and molecular analyzes were carried on cardiomyocyte-specific synemin-knockout mice (SynHKO) after 14 days of isoproterenol administration (30 mg/kg/day) by Alzet mini-pumps. Moreover, we investigated the response of human SYNM-KO induced pluripotent stem cells (iPSC) – derived cardiomyocytes to stimulation with isoproterenol (10 nM) or forskolin (10 μM), both activators of PKA.

After isoproterenol administration, SynHKO mice exhibited increased heart weight (+14%) and cardiac cell width (+26%), significant fibrosis, and left ventricular dysfunction compared to controls. Then, we generated human SYNM-KO iPSC lines using CRISPR/Cas9 system. They are able to differentiate into beating cardiomyocytes with desmin and cardiac troponin expression. The human SYNM-KO cardiomyocytes, stimulated for 7 days with isoproterenol, exhibit an increase of their area (+52%). No noticeable effect was observed in the control group. Measurements of the calcium flux and contraction kinetic 20 minutes after forskolin stimulations indicated that SYNM-KO cardiac cells reached calcium and contraction peaks faster with a 24% increase of calcium decay time and beat interval compared to control cells. Finally, a variation in the phosphorylation profile of PKA targets was observed in these cardiomyocytes stimulated by forskolin.These findings suggest a crucial role of synemin in cardiac remodelling and the loss of synemin-PKA coupling could trigger these alterations.

Keywords: Synemin CRISPR-Cas9 Cardiomyopathy Isoproterenol iPSC

Effects of blood-brain barrier opening with ultrasounds combined to microbubbles on tau pathology and prion-like propagation

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Alzheimer's disease is the most frequent cause of dementia. To date, almost no disease-modifying therapeutics have proven clinical efficacy which might be related to the existence of a blood-brain barrier (BBB) that drastically restricts exchanges between central nervous system and peripheral fluids (e.g. penetration of drugs, clearance of toxics). BBB can be safely, transiently, and repeatedly opened using low-intensity pulsed ultrasounds with microbubbles (LIPU-MB). While beneficial effects of BBB opening have been shown in amyloid mouse models, its effects on tau pathology remain unclear. Our study aims at evaluating the effects of LIPU-MB BBB opening on tau lesions in transgenic P301S mice.

Five sessions (one per week) of LIPU-MB (at 0.2 or 0.3 MPa) were administered to 20 P301S mice to assess the effect on tau pathology. A subset of these mice received a stereotaxic injection of human tau-purified extract in the hippocampus to boost tau propagation before LIPU-MB. 15

P301S mice received a sham procedure. All mice were sacrificed one week after the last sonication session. Immunostaining of phosphorylated tau was compared between the groups. P301S mice sonicated at 0.2 MPa did not show any significant modulation of endogenous tauopathies. However, BBB opening at 0.2 MPa was able to reduce tau propagation in distant brain areas (piriform cortex, amygdala) of P301S mice with accelerated pathology (inoculated mice). Importantly, BBB opening at higher acoustic pressure (0.3 MPa) induced adverse effects (e.g. cerebral hemorrhages with foci of neuroinflammation) and concurrently a discrete but significant increase in tau burden.

BBB opening with 0.2 MPa LIPU-MB is able to reduce tau spreading in P301S mice. Sonication at higher intensities is associated with adverse effects. Further investigations are needed to elucidate the underlying mechanisms of action, but these results could have therapeutic impact for tau-driven neurodegenerative diseases.

Keywords: Alzheimer's disease; BBB opening; ultrasounds; microbubbles

Characterization of CD14, TLR4 and ICAM3 contribution to the circadian regulation of retinal phagocytosis

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The circadian elimination of photoreceptor outer segment (POS) by cells from the retinal pigment epithelium (RPE) is based on the recognition of phosphatidylserines, apoptotic eat-me signals displayed by their most aged extremities that are also recognized by macrophages. Cluster of Differentiation 14 (CD14), Toll Like Receptor (TLR4) and the InterCellular Adhesion Molecule 3 (ICAM3) are involved in the clearance of apoptotic cells by macrophages and are also expressed by RPE cells. We thus set out to characterize their potential participation in the regulation of the daily phagocytosis of photoreceptor outer segment (POS), alone or as co-receptors.

Candidate's participation in the regulation of the POS rhythmic phagocytosis and the receptor-receptor interaction in vitro are studied on the rat RPE-J cell line using inhibition assays (double siRNA tranfections, blocking antibodies) and immunofluorescence. In vivo, their circadian expression is studied using RT-qPCR and western blots on RPE/choroid tissues from control mice euthanized at different times along the light:dark cycle.

ICAM3 inhibition on POS has a direct effect on POS internalization, an effect not seen when we inhibited the receptor on RPE cells. We confirmed by immunolabeling assays that ICAM3 is not expressed by RPE cells. CD14 and TLR4 gene expression and function inhibition decreased POS phagocytosis. The same inhibition experiments on the macrophage J774 cell line have a different effect on POS phagocytosis, suggesting a tissue-specific role of the CD14-TLR4 interaction in the retina. Upon POS challenge, CD14 and TLR4 expression levels at the cell surface increase, receptors form clusters and directly co-localize with POS and with one-another at 3 and 5 hours of phagocytosis. In vivo, CD14 mRNAs display 2 peaks of expression at ZT3, just after the time of peak phagocytosis, and at ZT8, a downtime for phagocytosis.

Taken together, our current data suggest that CD14 is involved in POS phagocytosis by RPE cells as co-receptor to TLR4. Further in vitro and in vivo investigations are currently being performed in order to fully understand their exact participation to this crucial function and to identify the associated signaling pathways.

Keywords: retinal pigment epithelium, phagocytosis, CD14, TLR4, ICAM3, co-receptor

Modeling growth plate dynamics using hypertrophic chondrocytes to understand growth retardation in Silver-Russell Syndrome

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Parental imprinting is an epigenetic regulation mechanism (currently DNA methylation) that takes place in gametes and in the early stage of fetal life, leading to the differential expression of some genes (about 130 in human) depending on parental origin. The disruption of parental imprinting is involved in a dozen human pathologies called Imprinting Disorders (IDs). Silver-Russell syndrome (SRS) is a rare disease affecting 1 child/100 000. This disease impacts fetal and postnatal growth and metabolism, with long-term health consequences. This syndrome is due in 50% of cases to a loss of methylation at the paternal ICR1 (an imprinting control region) in the 11p15.5 chromosomal region leading to a decreased expression of an important gene for growth, Insulin Growth Factor 2 (IGF2). Diseases secondary to parental imprinting abnormalities, generally involving tissues that are difficult to access, are still poorly understood and require the development of relevant and innovative models for a better characterization

In this context, the development of a model of hypertrophic chondrocytes, cells involved in the growth of long bones is relevant to better understand the mechanisms underlying growth retardation in patients with SRS. To achieve this, induced Pluripotent Stem Cells (iPSCs) from controls and SRS patients will be differentiated into sclerotome, an embryonic structure derived from somitic mesoderm, then, into hypertrophic chondrocytes. The maintenance of methylation patterns in imprinting regions and successful iPSCs differentiation into hypertrophic chondrocytes were confirmed by RT-qPCR, immunohistochemistry and protein assays.

While this study has not yet demonstrated significant transcriptomic or histological differences between control hypertrophic chondrocytes and those from patients during differentiation, further RNA-sequencing investigations and hormonal stimulation may reveal alterations in growth-related signalling pathways and hormone sensitivity deficits. This new model could allow major advances in the understanding of IDs affecting growth and open the way to new therapeutics for patients.

Keywords: growth, parental imprinting, Silver-Russell Syndrome, chondrocytes, Stem Cells

Matrix Vesicle-driven mineralization in osteoarthritis.

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In osteoarthritis (OA), the cartilage extracellular matrix is the subject of pathological remodelling and the formation of calcium phosphate (CaP) minerals, which is accompanied by an advancement of the mineralization front in the deepest part of cartilage. The cartilage mineralization process mainly involves phenotypic changes of chondrocytes, which undergo a process of terminal differentiation and become hypertrophic. These chondrocytes produce matrix vesicles (MVs) in the extracellular space, which have been identified as the locations where CaP mineralization is initiated. MVs are, indeed, highly enriched with several proteins, particularly mineralizing enzymes, and are characterized by specific composition of their internal content (ions, proteins, RNA). However, the mechanism by which CaP mineral nucleates and grow via MVs remains debated in the literature. In this PhD project, MVs were isolated from human osteoarthritic cartilage and characterized, in terms of size, protein and nucleic acid contents and enzymatic activities. Moreover, real-time mineralization of MVs was monitored by atomic force microscopy (AFM) to probe the evolution of their morphologies and their nanomechanical properties.

Keywords: Mineralization, osteoarthritis, Matrix vesicles

Study of the Ankle Ligamentoplasty Process: Towards Novel Biomarkers

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Ankle sprain is a common pathology that generates significant public health costs, with chronic instability as its main complication occurring in nearly 20% of cases. Surgical treatment may be indicated in cases of failed medical treatment, aiming to stabilize the ankle and prevent the development of osteochondral lesions leading to tibiotalar arthrosis. However, the timing of surgical intervention remains variable. Moreover, postoperative management lacks consensus, with differing strategies and variable return-to-sport timelines due to a lack of objective measurement tools assessing the ankle's ability to withstand athletic stresses.

This thesis has a dual objective. Firstly, it aims to develop in vivo markers for analyzing ligamentization processes in patients undergoing anatomical ankle reconstruction using MRI, elastography, and synovial fluid analysis. Secondly, an animal model will be developed to finely analyze tissue modifications (cartilage, bone, synovial fluid) following ligament rupture and associated ligament repair. In vivo marker development involved adapting the Signal/Noise Quotient (SNQ) for ankle ligamentoplasty monitoring, alongside validating elastography for graft maturation tracking. A prototype to impose ankle stresses is being developed. Additionally, synovial fluid protein profiling using Raman spectroscopy aims to provide another in vivo marker for graft maturation and ankle stability assessment. Experimental work includes establishing a chronic lateral ankle instability rat model. Once validated, comparisons between early and delayed repairs will be made. SNQ adaptation (SNQA) and elastography are valid for graft ligamentization assessment. Elastography indicates graft stiffness reduction over time, remaining stiffer than native ligament. A prototype for ankle laxity diagnosis and ligamentoplasty monitoring is under development.

This three-year study validated non-invasive in vivo markers for ligamentoplasty evaluation and monitoring. Additionally, it established a rat ILCC model for future exploration of ankle-related pathologies and treatments.

Keywords: Instabilité chronique de la cheville, ligamentoplastie, reconstruction anatomique, élastographie, SNQA

Evaluating synergistic dynamics of Metformin and Simvastatin on Ovarian Cancer Cells

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Ovarian Cancer (OC) stands as the most lethal gynecological malignancy, presenting an urgent clinical challenge in the quest to improve response rates. One approach to address this challenge is through drug repurposing, exemplified by the investigation of metabolic-modulating drugs such as Metformin (MTF) and Simvastatin (SIM). This study aims to explore the molecular mechanisms contributing to the potential synergistic anti-cancer effects between MTF and SIM on ovarian cancer cells.

We assessed the effects of the combination on the proliferation and viability of two cell lines OVCAR-3 and SKOV-3. IC50 concentrations of MTF and SIM were determined using a proliferation assay, followed by subtoxic concentrations to explore the potential synergistic effects on the viability of both cell lines. Transcriptomic analysis was conducted on OVCAR-3 treated cells, and the findings were validated by assessing the expression levels of differentially expressed genes (DEGs) through real-time PCR in both cell lines SK-OV-3 and OVCAR-3.

Cytotoxicity analysis guided the selection of treatment concentrations as such MTF 10 mM and SIM 5 μM. The combined treatment of MTF and SIM demonstrated a synergistic inhibition of proliferation and viability in both cell lines. In OVCAR-3, exclusive identification of 507 DEGs was seen in the combination arm. Upregulation of FOXO3, RhoA, and TNFα, along with downregulation of PIK3R1, SKP2, and ATP6V1D levels, was observed in OVCAR-3 treated cells. Real-time PCR validation confirmed the consistency of expression levels for the mentioned DEGs.

Our data strongly supports the presence of synergy between MTF and SIM in OC cells. The combination's effect is associated with the dysregulation of genes in the key regulators AMPK and mTOR alongside other interconnected pathways.

Keywords: ovarian cancer, cell metabolism, drug repurposing, synergy analysis, transcriptome profiling

Immunomodulation du neurolupus par la cannelle dans un modèle murin de lupus induit par l'imiquimod

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La physiopathologie du neurolupus est complexe et reste difficile à prévenir. La cannelle possède des propriétés neuroprotectrices. Nous investiguons le rôle de la cannelle comme agent thérapeutique du neurolupus dans un modèle expérimental induit par l'imiquimod.

Des souris femelles C57BL/6J sont divisées en 5 groupes : contrôle (S), contrôle traité par la cannelle (SC), lupus (L), lupus traité (LC), et lupus traité avant et pendant l'induction (CLC). L'induction est réalisée par l'application cutanée d'imiquimod, sur l'oreille droite, 3 fois par semaine pendant 6 semaines. Cinnammomum Cassia est administrée à la dose orale quotidienne de 200 mg/kg. À la 6ème semaine, des tests comportementaux sont réalisés. Après le sacrifice, des coupes histologiques sont effectuées sur le rein et le cerveau, avec dosages d'expression protéique des jonctions serrées sur l'intestin et le cerveau, TLR7 et NLRP-3 sur le cerveau.

L'induction du lupus est confirmée par la présence d'une glomérulonéphrite. L'expression des jonctions serrées intestinales est significativement réduite dans les groupes lupiques mais rétablie avec le traitement par la cannelle. Une augmentation des comportements dépressifs et anxieux est constatée dans les groupes lupiques avec une atteinte cognitive ; ces altérations sont significativement améliorées dans le groupe CLC. L'histologie hippocampique montre une condensation neuronale de la chromatine et augmentation de TLR7 et NLRP3 dans les groupes lupiques ; ces signes étaient moins prononcés dans le groupe CLC. Une redistribution des jonctions serrées de la bordure inter-cellulaire vers le compartiment intracytoplasmique a été mise en évidence en immunofluorescence, indiquant un dysfonctionnement de la barrière hémato-méningée, rétabli dans le groupe CLC.

Notre étude démontre pour la première fois un effet préventif de la cannelle sur la dysfonction de la barrière hémato-méningée et la neuroinflammation. Cinnamomum cassia pourrait faire partie de l'immunomodulation du futur du neurolupus

Keywords: lupus, TLR-7, cinnamon, Tight junctions, brain

Elaboration of a valid engineered multispecies endodontic biofilm, and an in vitro evaluation of the antimicrobial activity of five calcium silicate based root canal sealers against it

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The aim of this study was the development of a complex multispecies endodontic biofilm using C. albicans, P. mirabilis and P. aeruginosa on a biofilm of E. faecalis that was cultivated earlier in different growth conditions. In addition of comparing the antimicrobial activity of five calcium silicate based root canal sealers against it.

Fifty two single rooted extracted human teeth and fifty two dentinal discs were mechanically prepared, sterilized, inoculated with E. faecalis and divided randomly into 8 groups where the substrate, the inoculation technique, the medium type, and the pre-treatment with collagen type I was variable. P. mirabilis, P. aeruginosa and C. albicans were added after 10 days of static incubation in 3 different sequences. After 16 days the biofilm was removed placed in sterile BHI and dissected. The bacterial count was evaluated and colonies were counted on different agars. The resulting biofilm was then placed on a 10mm round shaped blotting paper set on a metallic net in a 6cm petri dish with an orthodontic bend filled with the calcium silicate based root canal sealer in direct contact with the biofilm.

The monobacterial E. faecalis biofilm showed on day 14 of the culture, the highest values in all groups in root canals and when Type 1 collagen pre-treatment and glucose were used. In the group where P. aeruginosa was added directly after E. faecalis followed by C. albicans and P. mirabilis respectively the bacterial count showed a significantly greater number compared to the other groups. When it comes to calcium silicate-based sealers, in all tested groups, the total bacterial count has significantly decreased between day 3 and day 14 with no statistically significant differences among the different sealers' groups at all time points.

To increase the clinical success rate, we should learn more about the traits and properties of bacteria, their biofilms, as well as the changes in the root canal environment.

Keywords: Endodontic biofilm, Multispecies Biofilm, Endodontic Microbiology, Calcium Silicate Sealers, E. faecalis.