ANNUAL CONFERENCE

11th of december
Domaine du Haut-Carré
Talence

Département de recherche
SBM | Sciences biologiques et médicales
université de BORDEAUX

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As in previous years, the annual Biological and Medical Sciences Day offers you the opportunity to create and strengthen collaborative projects and interactions between the various research laboratories, notably through selected oral presentations representative of the many lines of research developed in our department.

The best flash posters and posters will be awarded prizes by the members of the jury at the end of the day. Don’t hesitate to vote using the QR codes.

Two guest speakers, Valérie Delague and Luisa Miranda Figueiredo, will present their work during the day.

Finally, there will also be a session dedicated to sustainable development.

We wish you all a wonderful Biological Sciences Department Day.

Anne Cayrel, Iris Hasantari, Candice Menegon, Alexandra Moisand, Yasmine Pobiedonoscew, Alexandra Prevôt, Muriel Priault, Loïc Rivière, Manuel Rojo, Claire Rouy.

Program

9h00 Opening of the day
Alain-Pierre Gadeau

9h10 Presentation of the CASDEN
Isabelle Lefebvre

9h15 Platform presentation
Béatrice Turcq

9h30 Scientific session
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Domitille Chalopin-Fillot | *IBGC*
«Identification of monocyte states and their differentiation paths using a single-cell myelopoiesis atlas»
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«Complex roles of Nucleic acid specific TLRs in lupus pathogenesis»
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«Timely chromatin invasion during mitosis governs Prototype Foamy virus integration site selection and infectivity»

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«From gene to treatment: translational research in the field of Inherited Peripheral Neuropathies»

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13h00 Poster session 1

14h00 Plenary session

Chairman: Loïc Rivière

Luisa Miranda Figueiredo | *Instituto de Medicina Molecular, Lisbon, Portugal*

«Learning new biology by studying an ancient parasite»

14h45 Sustainable development session

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«La conversion écologique des Français. Contradictions et clivages»

15h15 Coffee break and poster session 2

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«Complications associated with hematopoietic aging»

Antonio Pagano | *BRIC*

«Molecular Mechanism of Cannabinoids in Glioblastoma: A New Insight»

Sara Kassir | *MRGM*

«Identification of obesogens using zebrafish larvae as a model»

Claire Almyre | *IBGC*

«Drug repurposing for mitochondrial disorders»

17h00 Award winners and closing conclusion

Katia Boniface

Laura Desbourdes | *BRIC*

«Modeling the human bone marrow niche to explore treatment resistance in acute myeloid leukemia»

Desbourdes L[1], Rouy C[1], Desmares-Romain E[1], Villacreces A[1], Guitart A[1], Pasquet JM[1], Vigon I[1], Dumas PY[1,2], Desplat V[1].
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[2] Service d'Hématologie et Thérapie Cellulaire, CHU Bordeaux, Bordeaux, France.

The treatment of acute myeloid leukemia (AML) has undergone a veritable boom with the advent of targeted therapies, and in particular the use of tyrosine kinase inhibitors (TKIs) for the treatment of AML with mutations of the tyrosine kinase receptor FLT3. Despite this therapeutic advance, these leukemias are still associated with a poor prognosis and frequent relapses. Studying the resistance mechanisms to TKIs and the persistence of leukemic stem cells (LSCs), responsible for relapse, is the key to improving the therapeutic management of patients with relapsed or refractory FLT3-mutated AML. Our team has identified novel splice variants of this receptor that may be involved in these resistance mechanisms. To characterize these new variants, we are exploring their oncogenic potential by studying their cellular localization, pre-leukemic potential and leukemic aggressiveness *in vivo*, comparing them with the known mutated FLT3 forms. Our team has also demonstrated that some of the resistance mechanisms to TKIs are dependent on the hematopoietic and hypoxic microenvironment, and that this induced-resistance can be overcome by targeted therapy. In order to explore the protective interactions of the microenvironment with leukemic cells and LSCs in the context of TKI therapies, it is essential to get as close as possible to the reality of the patient's bone marrow. To this end, we will optimize some previously published models to reconstitute, *in vitro* and *in vivo*, a humanized bone marrow niche able to welcome leukemic blasts from patient samples or from cell lines and to maintain the LSC population in mice. In this new project, these « mini leukemic bone marrows » will be treated with different anti-FLT3 TKIs and conventionnal chemotherapies, either as monotherapy or in combination, in order to identify new therapeutic targets. This study will potentially enable the development of new therapeutic strategies for patients with relapsed or refractory FLT3 AML.

Domitille Chalopin-Fillot | *IBGC*

«Identification of monocyte states and their differentiation paths using a single-cell myelopoiesis atlas»

Chalopin D., Specque F., Boyer T., Giraud J., Larmonier N., Saleh M., Nikolski M.

Hepatocellular carcinoma (HCC) is an inflammation-associated cancer caused by factors such as viral infections, alcohol abuse or obesity. Despite a significant therapeutic advance in the treatment of HCC, ~75% of patients do not respond to immunotherapies for unclear reasons. To gain a better understanding of this resistance, it is important to consider the expansion of suppressive myeloid cells, a hallmark of chronic inflammation and cancer. However, their heterogeneity and their differentiation process are not fully resolved, potentially underlying immunotherapy resistance. Depicting a detailed range of transcriptomic programs, single cell technologies enable the improvement of our knowledge of cell state

granularity. The main objectives of this study are to 1) to refine monocyte state annotation and 2) to reach a deep overview of the regulatory programs driving immunosuppression in HCC. Using more than 100 samples from twelve single-cell datasets from bone-marrow, blood and liver (healthy and tumoral) mimicking the myelopoiesis lineage, we aim to establish important and novel computational approaches to build a robust cell atlas. The key findings will encompass a curated list of potential (coding and non-coding) RNA candidates involved in the acquisition of suppressive functions, paving the way for exploration of their therapeutic potential in RNA-based interventions.

Claire Leibler | *ImmunoConcEpT*

«Complex roles of Nucleic acid specific TLRs in lupus pathogenesis»

Toll like receptor (TLR) 7 and 9, endosomal sensors for RNA and DNA, are key mediators of lupus autoreactivity. Although previously considered homologous, they have opposing effects on lupus severity: TLR7 exacerbates disease while TLR9 protects when expressed in B cells. How, then, do they mediate opposing effects in lupus disease? We recently suggested that TLR9 has a MyD88-independent regulatory signaling pathway. We therefore hypothesized that differences in signaling qualities of the two TIR domains, which share only 45% homology, could be responsible for the opposite outcome on disease. To test this, I created a chimeric TLR9 molecule, called 997 (TLR997), in which the TLR9 signaling-TIR domain was replaced with TLR7 TIR domain. TLR997 was introduced by CRISPR/Cas9 in the endogenous TLR9 locus of TLR7-deficient lupus-prone mice. Preliminary results have shown that lupus disease is exacerbated in TLR997 compared to the native TLR9 (TLR999) mice (all TLR7-/-), proving that TLR7 and TLR9 TIR domains indeed have different signaling properties.
We now aim to characterize the functional differences between the TIR domains using our unique system. We will assess the B cell intrinsic effects of TLR9 and TLR7 TIR domains on B cell differentiation *in vivo*, using a 3-way bone marrow-chimera system, and *in vitro*. Using biochemical approaches, we will study if TLR7 and TLR9-TIR differs in the triggering of MyD88 signaling and/or in the binding of novel partners that would induce pro-inflammatory signals for TLR7 or negative regulation for TLR9.
The finding that TLR9 and TLR7 TIR domains signal differently is novel, changes the paradigm and opens exciting avenues to develop therapeutic targets in lupus. It will also have major implications beyond lupus for fundamental TLR and innate immune biology.

Paul Lesbats | *MFP*

«Timely chromatin invasion during mitosis governs Prototype Foamy virus integration site selection and infectivity»

F. Lagadec[1,2], P.Singh[3,4], D. Lindemann[5], A. Engelman[3,4], P. Cherepanov[6,7], V. Parissi[1,2], P. Lesbats[1,2]

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Selection of a suitable chromatin environment during retroviral integration is a tightly regulated and multilayered process that involves an interplay between viral and host proteins. Interaction between the Spumaretrovirus Prototype Foamy virus (PFV) Gag and cellular chromatin has been described as a major determinant for integration site selection. Crystal structure of Gag Chromatin-Binding Site (CBS) bound to a nucleosome revealed an interaction with histone H2A-H2B acidic patch via a highly conserved arginine anchor residue. However, the exact molecular mechanisms involved during chromatin capture for an optimal integration site selection are still obscure.

In this study, we investigated the kinetic of Gag-chromatin interaction and proviral integration during PFV infection of synchronized cells. Using viruses harboring substitutions in Gag CBS, we showed that affecting its affinity for the nucleosome induces an untimely chromatin tethering during mitosis leading to a decreased infectivity and a variant-specific redistribution of the integration sites. We also provide mechanistic evidences for the improper chromatin binding as the result of Gag inability to displace H4 tail from nucleosome acidic patch of highly condensed mitotic chromatin. These data suggest that the mitotic chromatin landscape during Gag–nucleosome interaction dictates integration site selection and that Spumaretroviruses evolved a strong chromatin binding capacity in order to invade host chromatin in a timely manner for optimal replication.

Plenary session

Valérie Delague | *Institut Marseille Medical Genetics, France*

«From gene to treatment: translational research in the field of Inherited Peripheral Neuropathies»

Flash presentation

Léa Pechtimaldjian | *BRIC*

«Unraveling vascular and telocytes remodeling in cutaneous infantile hemangiomas using large-volume 3D imaging»

Infantile hemangioma (IH) is the most frequent tumor in newborns, occurring in up to 1 in 10 births. This benign vascular tumor exhibits a fast-growing phase followed by a slow and gradual involution. Ten years ago, propranolol emerged as a remarkably efficient drug to accelerate its regression. While mechanisms of actions remain largely unknown, previous work of the team has demonstrated the key role of a new stromal cell in the pathogenesis and propranolol response: the telocyte (TC). Given the pivotal role of TCs in cellular communication and organization, we hypothesized that TCs could participate in IH involution by inducing a vascular remodeling process, further potentiated by propranolol. In this study, we aim to investigate the vascular organization of IH through a comprehensive architectural analysis of patient resections, classified according to the tumor state and the treatment. Achieving deep and accurate visualization of remodeling necessitates large volume imaging of tissue. However, working on thick samples require tissue-clearing, a technique initially developed for mouse brains. Nonetheless numerous adaptations are required for skin, a highly pigmented and matrix-dense tissue. Accordingly, we have developed an optimized tissue-clearing protocol named Skin-iDISCO+, specifically tailored for skin clearing, which was previously insufficiently addressed. With Skin-iDISCO+ and compatible antibodies, we successfully identified a limited number of tortuous vessels within tumors. Results showed that the reduction of the tumor vascular burden was correlated with vessel straightening. Additionally, we observed distinctive arrangement of TCs around lesional capillaries. They either form sheets or branching meshwork covering vessels. These arrangements appear as being associated with different tumor states and vessel straightening. These findings indicate that natural or propranolol-induced IH involution involves a dynamic TCs reorganization, leading to vascular remodeling. A deeper understanding of propranolol mechanisms holds promise to explore therapeutic prospects for other vascular tumors with unmet clinical needs.

Mohammad El Kadri | *MFP*

«Glycerol, abundantly present in mammalian tissues, induces differentiation of trypanosomes into the stumpy-Glyc transmission-competent forms»

Trypanosoma brucei is an extracellular parasite responsible for African trypanosomiasis, exhibiting a complex life cycle in an insect vector (procyclic forms, PCF) and a mammalian host (bloodstream forms, BSF). Survival in diverse environments requires morphological and metabolic adaptations. BSF trypanosomes were believed to exclusively thrive in mammalian fluids, particularly blood rich in glucose, as their sole carbon source for central carbon metabolism and ATP production. However, recent studies have shown that BSF trypanosomes can grow efficiently in the absence of glucose if supplied with glycerol, and most parasites reside in the extravascular compartment of the skin and adipose tissue, where adipocytes release substantial amounts of glycerol from lipolysis and glycolysis. Hence, we hypothesized that glycerol plays a novel role in African trypanosome biology, with interactions between adipocytes and extravascular trypanosomes potentially granting a selective advantage to the parasites in mammalian hosts. Our findings demonstrate that

when glycerol is metabolized, it induces the differentiation of proliferative slender BSF into growth-arrested or slow-growing forms similar to stumpy BSF (forms preadapted to the insect environment) that can efficiently differentiate to the PCF insect stage, implying a crucial role of glycerol in the transmission of the parasite from the mammalian host to the insect vector. It was examined by glycerol kinase and aquaporin null mutants, which failed to differentiate into stumpy BSF in the presence of glycerol. These results support our contention that glycerol, produced by adipocytes, influences trypanosome metabolism, prompting differentiation for transmission to the insect vector.

Margaux Laisne | *BMC*

«Serglycin at the Glia Limitans, a key player of neuro-inflammation pathophysiology»

Margaux Laisné[1], Elsa Veyrieres[1], Pierre Mora[1], Célia Bourguignon[1], Alain-Pierre Gadeau[1], Marie-Ange Renault[1], Candice Chapouly[1]
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Introduction: During neuroinflammation, astrocytes undergo morphological and molecular changes called “astrogliosis” enabling them to communicate with endothelial cells at the blood brain barrier (BBB) by producing pro-inflammatory factors. To characterize astrocytes signature during astrogliosis, we performed a RNA sequencing on “basal” (hBA) versus “reactive” human astrocytes (hRA) and identified Serglycin (SRGN), a proteoglycan, as highly expressed by hRA.
Objective and hypothesis: Our goal is to unravel the contribution of astrocytic SRGN to neuro-inflammatory behavior. Our hypothesis is that SRGN promotes glial scar formation and BBB breakdown.
Methods: *In vitro*, we transfected hbA with control siRNAs or SRGN siRNAs (siSRGN) before inducing astrogliosis with IL-1β treatment. Cell lysates and conditioned media were then harvested to perform transcriptomic and proteomic analysis. In parallel, Human Brain Microvascular Endothelial Cells (hBMECs) were incubated with hRA conditioned medium for 6 hours to perform transcriptomic analysis.
Results: siSRGN treated hRA present a disturbed actin cytoskeleton with stress fibers assembly and an altered astrocyte morphology with lesser protrusions associated to a slower and disorganized migration and decreased expression of the tight junction Claudin1. Therefore, SRGN seems to play an important role in glial scar formation.
On the other hand, siSRGN treated hRA secrete less pro-inflammatory factors notably IL-6 and IL-8, which are trapped in the golgi apparatus, and chemokines ligands such as CCL5 and CCL7. Moreover, conditioned medium of hRA treated with siSRGN modulates endothelial activation markers in hBMECs. Therefore, SRGN seems to promote and facilitate astrocyte’s inflammatory cytokine secretion which influence BBB activation.
Discussion: Collectively, our results demonstrate that SRGN in hRA drives astrogliosis properties, during neuro-inflammation, involved in glial scar formation and BBB homeostasis.

Johann Kervadec | *IBGC*

«Bcl-xL deamidation: a target against platelet aging ?»

Deamidation is the post-translationnal modification removing the amino-group of the lateral chain of Asparagine and Glutamine residues. It can occur in virtually any proteins. It is a spontaneous hydrolysis which doesn't require any enzyme. Consequently, deamidation of Asparagines leads to the production of Aspartate and Iso-Aspartate residues, which modifies the global charge of the protein and can alter its folding and function. The occurrence and kinetics of this reaction depend on both intrinsic parameters such as the n+1 residue, the local structure, and extrinsic parameters such as temperature, ionic strength and pH. Our team focuses on the protein Bcl-xL, an anti-apoptotic member of the Bcl-2 family. We discovered that Bcl-xL undergoes a step-wise deamidation on two Asparagines located in its unstructured loop. Deamidated Bcl-xL builds up with time in cells, which we proved instrumental to determine the age of circulating platelets. Platelets are anucleated blood cells playing an essential role in blood clotting. Their lifespan is around 10 days in humans. To overcome thrombocytopenia, a decrease in the platelet count in the blood stream, platelets can be transfused. However, because of their short shelf life, platelet concentrates are not stored for more than 7 days. Platelets respond to apoptosis controlled by Bcl-2 family protein, and survive as long as the pro-apoptotic protein Bak is kept inactive by the anti-apoptotic protein Bcl-xL. The mechanism by which Bcl-xL disengages from Bak is still unknown. We currently investigate whether deamidation is responsible for platelet termination and test whether Bcl-xL deamidation is the mechanism releasing Bak and inducing platelet apoptosis. If this is the case, a direct application will be the screening of compounds that delay Bcl-xL molecular aging and maintain its function in platelet concentrates, thus improving transfusion efficiency.

Elina Mercier | *MRGM*

«Variants of *OCA2* that cause exon skipping»

Elina Mercier[1], Vincent Michaud[1,2], Angèle Sequeira[1], Eulalie Lasseaux[2], Claudio Plaisant[2], Benoît Arveiler[1,2], Sophie Javerzat[1]

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Genetic diagnosis of patients with albinism is essential to adapt patient care and to offer genetic counselling to families. To date, around 30% of patients remain genetically unsolved, with a significant proportion (60%) presenting at least one rare variant of unknown significance (VUS) in one of the 20 known albinism genes.
In a previous study, we looked at a series of VUS from patients with suspected oculocutaneous albinism 2 (*OCA2*) that mapped in or around exon 10 of the *OCA2* gene. This exon is sensitive to skipping during splicing leading, in healthy subjects, to background levels of non-functional transcripts deleted from exon 10. By combining functional approaches (minigene assay, characterisation of transcripts from skin biopsies) we showed that some VUS in/around exon 10 could significantly increase its skipping and result in a defect in functional protein production sufficient to cause pathogenicity. In particular, several intronic and synonymous variants significantly increase exon 10 skipping, enabling us to classify them as pathogenic (Michaud et al., PCMR 2023).
We are extending this study to the characterization of sequences that control splicing of *OCA2* exon 10. First, we assess the effect of these rare variants when they are in cis with benign variants such as the common c.1065G>A;p.(Ala 355=), a predominant SNP in light-

skinned European populations. The identification of a haplotype with an additive effect on the exon 10 skipping will improve prediction tools. Second, we compare the human and murine sequences by minigene assay. Indeed, in mice, exon 10 of *Oca2* is not sensitive to skipping. We therefore select the few non-homologous nucleotides between the two species to test their ability to control splicing using minigenes. This should help identifying critical sequences of splicing regulation and facilitate genetic diagnosis.

Ribal Merhi | *ImmunoConcEpT*

«Impact of type-I interferons on melanocytes phenotype and function in vitiligo»

Vitiligo is an auto-immune skin depigmenting disease characterized by the loss of epidermal melanocytes, the skin pigmenting cells. Previous studies have shown that melanocytes in vitiligo are associated with an increased level of oxidative stress leading to the expression of senescence-associated genes. Moreover, our data revealed that type I interferon-related pathways are consistently increased in vitiligo perilesional skin. However, the role of type I interferons is not fully understood. My Ph.D. project aims to decipher the impact of type I interferon (IFN-a and IFN-ß) on melanocyte phenotype and function during vitiligo pathogenesis. Preliminary transcriptomic analysis by scRNAseq on perilesional skin of patients with vitiligo shows increased IFN-I signature in most skin cellular subsets, including melanocytes. Immunofluorescence assays shows that the expression of interferon-alpha receptor (IFNAR) by melanocytes is increased in perilesional skin of active vitiligo patients as compared to healthy controls and patients with stable disease. The expression of senescence markers such as cyclin-dependent kinase inhibitors p16INK4a and p21CIP/WAF1 is also increased in vitiligo skin as compared to healthy skin. Our in-vitro results show that keratinocytes stimulated with Toll-Like Receptor 3 agonists produce IFN-ß. In addition, out experiments show that melanocytes express a functional IFNAR. IFN-a and IFN-ß treated melanocytes show cell cycle arrest, increased expression of Senescence Associated Beta-galactosidase, phosphorylated-p53, p16INK4a, and p21CIP/WAF1. Furthermore, levels of major components of the senescence-associated secretory phenotype are increased in the presence of type I IFNs. Additionally, our RT-qPCR results suggest that neither IFN-a nor IFN-ß impact melanogenesis (DCT and TYR, coding for two key enzymes in melanogenesis, Dopachrome Tautomerase and Tyrosinase respectively) and cell adhesion (CDH1, coding for E-cadherin, the major melanocyte to keratinocyte adhesion molecule). Taken together, our results suggest that type I interferons induce a senescence-like phenotype in melanocytes.

Plenary session

Luisa Miranda Figueiredo | *Instituto de Medicina Molecular, Lisbon, Portugal*

«Learning new biology by studying an ancient parasite»

RNA modifications are important regulators of gene expression. In *Trypanosoma brucei*, transcription is polycistronic and thus most regulation happens post-transcriptionally. In this talk, I will explain how the extreme biology of *Trypanosoma brucei* allowed us to discover that certain poly(A) tails harbour N6-methyladenosine (m6A) and how this modification increases RNA stability. To our knowledge, this is the first identification of an RNA modification in the poly(A) tail of any eukaryote, uncovering a post-transcriptional mechanism of gene regulation. We will provide evidence that m6A levels change during cell differentiation and we will discuss ongoing efforts to identify m6A-readers.

Sustainable development session

Philippe Coulangeon | *Science Po Paris*

«La conversion écologique des Français. Contradictions et clivages»

L'omniprésence des injonctions à l'éco-citoyenneté a largement contribué à installer l'idée que la transition écologique serait essentiellement affaire de prise de conscience et de transformation de nos pratiques individuelles de consommation, de nos modes de déplacement et de nos manières d'habiter. Pourtant, les marges de manœuvre individuelles sont ténues et le postulat d'une relation de causalité entre préoccupation et action environnementale s'avère fragile.
Comme dans les autres pays occidentaux, la conversion des valeurs est largement réalisée au sein de la population française. La majorité des Français ont aujourd'hui intégré la réalité des enjeux environnementaux et climatiques. Toutefois, l'évolution des modes de vie n'emprunte pas le même chemin. Certaines pratiques écologiquement « vertueuses » se sont diffusées, mais l'évolution de nos modes de vie reste marquée par l'intensité de la demande de biens d'équipements électro-ménagers, relayés récemment par les équipements électroniques et numériques de loisirs tandis que la demande de véhicules automobiles, plus lourds, plus puissants, plus nombreux et plus souvent renouvelés s'intensifie et que nos habitudes alimentaires s'appuient tendanciellement sur des aliments plus transformés et de provenance plus lointaine.
Cette communication s'efforcera de montrer que la transition écologique ne saurait se résumer à l'adoption d'un catalogue de bonnes pratiques combiné à des choix techniques efficients. Elle suppose des arbitrages politiques qui touchent à des intérêts divergents et qui s'inscrivent dans les fractures sociales, économiques, culturelles et territoriales qui traversent la société française. Les défis de la transition écologique imposent à ce titre de repenser les termes de la question sociale. Alors que la justice sociale était pensée traditionnellement sous l'angle du partage des bénéfices de la croissance, l'urgence écologique soulève désormais d'abord la question du partage du fardeau de ses externalités.

Scientific session

Olivier Mansier | *BMC*

«Complications associated with hematopoietic aging»

Hematopoietic aging is associated with changes in the properties of hematopoietic stem cells together with a restriction of the hematopoietic stem cell repertoire. Among other alterations, this phenomenon is associated with epigenetic changes and the acquisition of genetic alterations.

The acquisition genetic alterations such as mutations in DNMT3A or TET2, or mosaic loss of the Y chromosome (mLOY) can increase the fitness of hematopoietic stem cells, allowing them to expand at the expense of normal hematopoietic cells. This leads to the production of a population of blood cells carrying the same genetic abnormality in the absence of hematological malignancy, a condition called Clonal Hematopoiesis (CH). CH is very common in the general population, increasing in prevalence with age, and reaching around 30% of people aged 60. Since their description in 2014, CH have been associated with a wide variety of diseases, mainly in the cardiovascular field. Since 2018, we have been investigating the involvement of CH and mLOY in the development of cardiovascular disease through the study of patient cohorts and animal models. In particular, we are interested in the association of CH/mLOY with the development of inflammation and fibrosis in the context of atherothrombosis and heart failure with preserved ejection fraction.

While the detection of CH can be considered as a capture of hematopoietic aging, the study of epigenetic alterations seems more accurate for this purpose. In particular, DNA methylation profiling has enabled the development of epigenetic clocks that reflect aging at tissue level.

To increase our ability to study cardiovascular and hematological complications linked to hematopoietic aging, we are currently developing research projects aimed at associating epigenetic aging with the development of thrombosis and myelofibrosis in the context of myeloproliferative neoplasms.

Antonio Pagano | *BRIC*

«Molecular Mechanism of Cannabinoids in Glioblastoma: A New Insight»

The cannabinoid receptor type-1 (CB1), one of the most abundant G protein-coupled receptors in the central nervous system, is pivotal in regulating neuronal transmission and various fundamental physiological processes. Intriguingly, CB1 can be functionally associated with mitochondrial membranes (mtCB1) in multiple types of brain cells, thereby governing their energy fluxes. Via mtCB1, cannabinoids reduce mitochondrial activity and lactate production. Furthermore, CB1 is expressed within glioblastoma (GBM), an aggressive and incurable form of brain malignancy characterized by robust metabolic adaptability. Extensive *in vitro* and *in vivo* investigations have demonstrated the substantial impact of cannabinoids on tumor growth and invasive properties; however, the exact mechanisms are not fully understood. Despite empirical observations highlighting the pro-apoptotic effect of CB1 agonists, their role in mitochondrial functions and in cancer cell metabolism has remained an area of limited exploration. Preliminary findings suggest the presence of mtCB1 within patient-derived GBM cells, underscoring its potential as a compelling target for disrupting cancer cell metabolism and crucial actor in the anti-glioma effect of cannabinoids. Given its capacity to modulate both oxidative phosphorylation and glycolysis, mtCB1 emerges as an intriguing candidate for therapeutic intervention within the context of GBM and its complex metabolic environment.

Sara Kassir | *MRGM*

«Identification of obesogens using zebrafish larvae as a model»

Sara Al Kassir, Anja Knoll-Gellida, Théo Mercé, Laure M. Bourcier, Sandra Pedemay, Magalie Soares and Patrick J. Babin
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Obesity is defined as a public health disease in which abnormal excessive fat accumulation causes health adverse effects. One proposed factor to the rise in obesity is the exposure to endocrine disruptors acting as obesogens. These obesogens affect white adipose tissue (WAT) directly by altering the adipocytes number, size and/or function or indirectly through other organs. Despite the rise in *in vitro* models, there is a need for alternative integrative models to assess obesogens in a physiologically relevant context. Thus, an optimization of a protocol called the zebrafish obesogenic test (ZOT) was conducted to become an OECD test for *in vivo* screening of obesogens and anti-obesogens targeting white adipocyte size. This short-term assay evaluates the dietary composition and/or environmental contaminants effects on adiposity. Zootechnical guidelines including larvae rearing, feeding and selection are provided. An optimized adipocyte staining with Nile Red before and after chemical exposure is delivered. Finally, a defined guide for fluorescence microscopy imaging and automatic image analysis via ZOT analysis software are provided using the anal fin ray subcutaneous adipose tissue as a suitable sublocation. Having in hands an optimized ZOT, selected chemicals were evaluated, first for their systemic toxicity and then for their potential obesogenic effect. Three of them were demonstrated as obesogens which are able to induce a thrifty phenotype by inhibiting, under fasting, fat mobilization or even by promoting its storage in WAT in dose-response manner. Moreover, chemical analyses were performed to study chemicals stability in media and their concentration inside larvae. Furthermore, to understand their mode of action (MOA) on larvae adiposity, omics analyses

and a spontaneous locomotion test were performed. The results demonstrated that a decrease in energy expenditure associated to larval locomotion was not sufficient to explain the MOA of the identified obesogens

Claire Almyre | *IBGC*
«Drug repurposing for mitochondrial disorders»

Mitochondrial diseases are diverse and pleiotropic, severe and largely untreatable. To help the discovery of candidate therapeutic molecules, we developed a screening method using yeast Saccharomyces cerevisiae models of mitochondrial disorders. Among these is the Barth syndrome (BTHS), a rare X-linked cardiomyopathy caused by mutations in a nuclear gene (TAZ) that encodes an acyltransferase (called Tafazzin) involved in the maturation of Cardiolipin (CL). This is a phospholipid with multiple functions in mitochondria that optimize oxidative phosphorylation (OXPHOS)-dependant production of the energy-rich ATP molecule. We isolated several FDA-approved compounds able to improve mitochondrial function in a yeast model of BTHS. One, Ebselen (EBS), holds promise in the treatment of Meniere's disease and bipolar disorders due to its antioxidant and inositol monophosphatase inhibitory properties. EBS proved to be active also in cells derived from BTHS patients and in a murine model of this disease. We provide evidence that the mechanism responsible for the rescuing activity of EBS in cardiolipin-remodelling deficient cells possibly results from a partial inhibition of cytosolic translation. Remarkably, we found that EBS has the capacity to rescue a large panel of other yeast and human cellular models of mitochondrial disorders.

Poster session

P1 - TORC1 dependent control of fission yeast cohesin
Besson Dorian

Cohesin is an ATP-powered molecular machine that mediates DNA tethering. Cohesin complexes build DNA loops in cis and hold together the sister chromatids in trans with essential roles in chromosome structure, segregation and repair. Cohesin functions require the cohesin loader, Mis4 in fission yeast (NIPBL in human), which binds to cohesin and stimulates its ATPase activity. How cells modulate cohesin activity and functions are barely understood. The TOR kinase is a master regulator of cell growth and metabolism, which is activated in response to intra- and extracellular signals, including nutrients, growth factors, hormones and cellular energy levels. TOR signaling is frequently dysregulated in cancers. Cohesin is also one of the complexes most frequently altered. We provide genetic and biochemical evidence linking TORC1 to Cohesin. A genetic screen for regulators of Mis4 yielded several components of the TORC1 complex. TORC1 mutants rescue the thermosensitive growth, cohesin binding to DNA and chromosome segregation defects of Mis4-G1487D. TORC1 components co-purify with cohesin and its loader Mis4. Label-free mass spectrometry analysis revealed that the Psm1 subunit of cohesin and Mis4 display TORC1-dependent phosphorylation sites and phospho-mutant analysis is consistent with the existence of a TORC1-cohesin signaling pathway.

P2 - Emergence of Ceftolozane/tazobactam resistance in Pseudomonas aeruginosa
Bientz Léa

Ceftolozane/tazobactam (C/T), a novel cephalosporin/ß-lactamase inhibitor combination, is a last resort treatment for patients with multidrug-resistant Pseudomonas aeruginosa infection. During a 13 months period between November 2017 and November 2018, we monitored the emergence of C/T resistance after treatment in order to characterize resistance mechanisms. Among the 60 patients treated by C/T, 9 (15%) carried at least one resistant isolate. We explored the ampC gene encoding the chromosomal cephalosporinase of P. aeruginosa. This gene sequencing revealed 7 different modifications that could lead to C/T resistance. Cloning experiments using the pUCP24 vector and PAO1 ?ampC strain, demonstrated that these modifications increased C/T MICs from 4 to 128 fold compared to the isogenic sensitive strain. The ampC genetic background didn't seem to impact the MIC. However, the level of resistance obtained with clinical strains presenting the same mutations may vary, suggesting that other mechanisms may be involve in C/T resistance. In some patients, several resistant isolates were detected (up to three), suggesting that some isolates may mutate more easily. Moreover, for most strains, C/T resistance restored the susceptibility to other ß-lactams especially imipenem. Most of ceftolozane/tazobactam-resistant strains were susceptible to imipenem/relebactam (93%) and cefiderocol (87%). Occurrence of mutations in the active site of AmpC plays the major role in the acquisition of C/T resistance. Nevertheless, difference exists between C/T MICs obtained from the clones compared to those presented by the correspondent clinical isolates, so NGS analysis will allow us to study other genes that may explain the level of C/T resistance.

P3 - The oncogene Src controls invadosome formation through a spatial modulation of the eIF3 translation complex

Bonnard Benjamin

Tumor progression and cell invasiveness require degradation of extracellular matrix (ECM) induced by invadosomes. Src-induced invadosomes are dynamic F-actin based structures enriched with translational machinery including subunits of eucaryotic translation initiation factor 3 (eIF3). The individual depletion of subunit 3H and 3E reduced invadosome formation and degradation activity associated with lower translational level. The inhibition of Src activity reduces translational level by the regulation of eIF3 expression and PI3K/mTOR pathway and eIF4E/eIF4G interaction. We show that eIF4E/eIF4G interaction, initiating translation, is involved in invadosome formation and associated-degradation activity. We also demonstrate that eIF3-regulated mRNAs enriched into invadosomes (Igf2bp2, TKT) are mandatory for their formation and ECM remodeling. Furthermore, we highlight the specificity of identified signaling (Src, eIF3, Igf2bp2 and TKT) overexpression in tumor section from hepatocellular carcinoma (HCC) patients and the determinant role of this new molecular mechanism to acquire invasive phenotype in HCC cell line Huh7. These findings reveal a new molecular mechanism of Src-induced translation leading to invadosome formation leading to tumor progression.

P4 - Cardiac pericytes may regulate lipid metabolism in a sex dependent manner

Bourguignon Célia

Free fatty acids (FFA) are the main substrate of energy used by cardiomyocytes. They result in the lipolysis by lipoprotein lipase (LPL) of circulating triglycerides from VLDL or chylomicrons. Importantly, the bioavailability of FFA is critical, as both a lack and an excess of FFA are deleterious for the heart and may cause heart failure. Lipid metabolism is then extremely regulated. Angiopoietin-like 4 (ANGPTL4), a negative regulator of LPL, whose expression is induced by FFA, acts as a feedback mechanism in FFA uptake, thus limiting lipid accumulation in the heart. Our goal is to identify mechanisms that regulate ANGPTL4 expression in the organ. To do so, we first exposed male and female mice to a high-fat diet (HFD) to induce hyperlipidemia. Surprisingly, we found the HFD regimen increases Angptl4 mRNA expression in the heart of females but not in males, suggesting sex hormones may be involved in the regulation of ANGPTL4 in the heart. To test this, we submitted female mice, ovariectomized (OVX) or not, to the HFD regimen. Interestingly, ovariectomy increased Angptl4 mRNA expression in mice fed with a normal diet, whereas in mice fed with a HFD regimen, Angptl4 mRNA expression was lower in OVX females than in non-OVX mice. This result suggests that female hormones may modulate the cardiac response to hyperlipidemia. To identify the cell type producing ANGPTL4 in the heart, we isolated cardiomyocytes, cardiac endothelial cells, and cardiac pericytes and found Angptl4 mRNA expression was higher in pericytes. Finally, we treated cultured cardiac pericytes with palmitate and found that it strongly enhanced Angptl4 mRNA expression, identifying cardiac pericytes as potential actors in the regulation of lipid metabolism in the heart. Whether and how sex hormones may regulate Angptl4 expression in cardiac pericytes remains to be identified. Our work identifies cardiac pericytes as new potential regulators of lipid metabolism in the heart through ANGPTL4 expression and that the role of cardiac pericytes may be influenced by sex hormones.

P5 - Spatial exploration of hepatoblastoma tissue bioarchitecture by volumetric imaging, artificial intelligence and applied mathematics

Calovoulos Alexia

Context : Despite a detailed characterisation of cellular components constituting the tumour tissue, the architecture of this pathologic tissue and the biological elements governing its structural organisation remain elusive. Recently, our consortium reported the first investigation on the complete ultrastructure of human tumour xenograft tissue by high-resolution 3D electron microscopy.
Methods : We are currently analysing the internal organisation of 5 hepatoblastoma patient-derived xenograft tissues and hepatocarcinoma spheroids as models by serial block-face scanning electron microscopy (SBF-SEM) using an integrated workflow combining manual and automatic segmentations, artificial intelligence (AI) and applied mathematics.
Results : We digitally reconstructed an entire tumour sample with a blood capillary, a haemorrhagic area and different cell types with their respective organelles. We developed image processing algorithms based on structural parameters (size, convexity and texture) enabling the discrimination between tumour cells, immune cells and endothelial cells. Furthermore, we are assessing the correlation between the size of hepatoblastoma cells and the size of their nucleus, cytoplasm and mitochondrial mass. We are also investigating the influence of blood capillaries and haemorrhagic areas on the planar alignment, polarity and size of tumour cells. Additionally, we are evaluating the reliability of algorithms in recognising and segmenting similar biological elements in different samples or regions of interest and with spheroids.
Perspectives and conclusion: Thanks to our integrated workflow, we aim to perform intra- and inter-tumour sample comparisons, allowing a deeper understanding of the organisation of tissues. These findings could potentially unveil differences and similarities among samples from different patients, providing valuable insights for clinicians and facilitating personalized treatment approaches.
Summary and objective : This comprehensive investigation of hepatoblastoma tissue architecture using advanced imaging techniques, AI and image processing algorithms has the potential to contribute to the emerging onconanotomy field. The resulting bioarchitectural parameters could assist in sample stratification, prognosis, and personalized treatment decisions.

P6 - Investigation in yeast of the mitochondrial DNA mutation m.9035T>C detected in patients suffering from neuromuscular diseases

Charles Camille

The mitochondrial DNA (mtDNA) is a remnant of an ancestral prokaryotic genome that is necessary for the production in eukaryotes of enzymatic complexes that generate the energy rich adenosine triphosphate (ATP) molecule. Being exposed to reactive oxygen species, this DNA has a relatively high propensity to accumulate mutations. Evaluating the pathogenicity of these mutations may be difficult because they often affect only a fraction of the numerous copies of the mitochondrial genome, which is referred to as heteroplasmy. Furthermore, there is no reliable method for genetically transforming human mitochondria. The yeast Saccharomyces cerevisiae provides a convenient model for investigating the consequences of human mtDNA mutations in a defined genetic background. Owing to its good fermentation capacity, it can survive the loss of mitochondrial ATP production, its mitochondrial genome

can be manipulated and this yeast is unable to stably maintain heteroplasmy, leading to homogenous mtDNA populations in cells. Taking advantage of these unique traits, we investigated the functional consequences of the m.9035T>C mutation, repeatedly detected in families with hereditary spinocerebellar ataxias. This mutation is located in the ATP6 gene encoding the subunit a of the ATP synthase complex. This complex uses the potential energy of a proton gradient across the inner mitochondrial membrane to produce ATP from ADP and inorganic phosphate. We found that an equivalent of the m.9035T>C mutation severely affects ATP synthase function and yeast growth from non-fermentable carbon sources. Intragenic suppressors at the level of the m.9035T>C mutation or in another codon of the ATP6 gene have been selected and their biochemical properties have been characterized. In the light of high-resolution structures of ATP synthase, the results make it possible to propose a molecular mechanism by which the m.9035T>C mutation compromises human health.

P7 - HIV-1 transcription and latency in T-lymphocytes

Damour Alexia

HIV-1 latency is a major challenge towards patients cure. Indeed, reactivation of latent viruses is a source of rebound in case of therapy interruption. Two strategies of latent virus eradication are under investigation. The “block and lock” strategy aims to silence permanently all viruses, whereas the “shock and kill” approach aims to reactivate latent viruses by Latency Reactivating Agents (LRAs) leading to the elimination of the infected cells by the immune system or by the cytopathic effects of the virus. This approach is currently inefficient due to the stochasticity of HIV-1 reactivation. This stochasticity is caused by random switching of the promoter between ON and OFF states with regulation, which is largely unknown. To elucidate underlying mechanisms, we follow transcriptional dynamics of HIV-1 by live cells microscopy. Our approach is based on the bacteriophage MS2 Coat Protein (MCP), which binds specific stem-loop of RNA with high affinity. We have established T-lymphocyte cell lines with different integrations of HIV-1 reporter tagged with MCP binding sites, which express MCP fused to a fluorescent protein Staygold. MCP-Staygold binds the HIV-1 RNA reporter and allows to follow viral transcription in live cells. We have found that in T-lymphocytes, viral RNAs were produced in stochastic transcriptional bursts. The bursts kinetics was distinct for different integration sites with the duration of OFF promoter states that were mostly affected. In addition, the reactivation potentials of our clones after treatment with different LRAs, acting on chromatin compaction, transcription factors recruitment and T-cell activation, differed in numbers of responding cells and the strength of the promoter activation. Therefore, HIV-1 integration site affects latency reactivation. We will next investigate the features of the integration sites, which play a role in latency and the input of the promoter proximal pausing and the chromatin environment on the latency at different genomic locations.

P8 - Specific tissue features govern two distinct pathways driving cytokinesis

Deduyer Irène

Cytokinesis is essential to partition the cellular content into two daughter cells. This fundamental process occurs after sister chromatid segregation and relies on the assembly of an acto-myosin contractile ring at the cell equator. The constriction of the ring drives the ingression of the cleavage furrow. The small GTPase Rho1 and its activating RhoGEF (called Pbl in Drosophila) are essential for this process by driving ring assembly and constriction. The team has discovered that two Pbl isoforms, Pbl-A and Pbl-B act concurrently to control Rho1 activity during the asymmetric division of the Drosophila neuroblast. Furrow-enriched Pbl-A focuses Rho1 activity at the furrow to sustain efficient ingression, while Pbl-B pan-plasma membrane localisation broadens the zone of Rho1 activity, which is critical to adjust furrow position, thereby preserving correct daughter cell size asymmetry. These findings highlight how the use of two isoforms with distinct localisation make asymmetric division of the neural stem cell more robust. However, how these two distinct pathways for Rho1 activation participate in making cytokinesis more robust in other tissues is not known. We found that flies lacking Pbl-B are viable but the adults have a reduced lifespan, cognitive impairment and are male sterile. In contrast, flies lacking Pbl-A are fertile but exhibit severe embryonic and pupal lethality rate. These findings indicate that, while Pbl-A and B have redundant activities, the level of their contribution to cytokinesis is tissue-dependent. Using genetics and live imaging we will present how the Pbl-A vs Pbl-B pathway is favoured depending on specific tissue features.

P9 - 3D cell models to study liver pathophysiology : from healthy liver to NASH and HCC disorders

Delamarre Adèle

Hepatocellular carcinoma (HCC) is the most common liver cancer and a major public health problem. With an increasing incidence linked to obesity and diabetes, Non Alcoholic Steatohepatitis (NASH) is about to become the leading cause of HCC worldwide. However, there is no predictive marker of NASH-driven HCC and carcinogenesis mechanisms are still poorly understood. That is why modeling NASH disease and carcinogenesis is crucial to better understand underlying molecular pathways and find new therapeutic targets. 2D cellular models are too distant from liver complexity while murine models do not recapitulate the whole human disease. 3D cell models allow better disease modeling thanks to 3D cell interaction and better cell function. Existing 3D NASH models may be relevant but they are too expensive and difficult to handle for high-throughput use or gene manipulation. However, in the new era of -omics technologies, there is a growing amount of human data that need an *in vitro* screening before starting preclinical studies. In particular, we identified new interesting pathways by proteomic analysis of NASH patients' biopsies that may be involved in carcinogenesis and need to be characterized in a NASH model by mimicking keys proteins' dysregulation. So our goal is to set up a new 3D cell model for NASH that is easy to produce and to manipulate in order to investigate carcinogenesis through the functional characterization of pathways identified in our human data. To answer this challenge, we chose the HepaRG® cell line that is described as the closest to primary human hepatocytes but easier to grow. We used a spheroid model and a NASH induction medium enriched in fatty acids, glucose and LPS. We succeeded in developing an easy-to-handle model with long viability, retaining main hepatocytes functions and recapitulating main NASH features that is suitable for validation of proteomics data.

P10 - Spectre génotypique des patients atteints d'albinisme de Bamako (Mali).

Diallo Modibo

Albinism is a phenotypically and genetically heterogeneous condition characterized by a variable degree of hypopigmentation and by ocular features leading to reduced visual acuity. In Mali, no genetic study of patients with albinism has been carried out yet. In collaboration with the dermatology and ophthalmology teams at the Infirmerie Hôpital Militaire in Bamako and the Association for the protection of patients with albinism in Bamako we determined the genotypic spectrum of 23 patients. We sequenced the 20 known albinism genes. The index cases presented with a moderate or severe oculocutaneous albinism phenotype. OCA2 was the most frequent form (17/23 patients, 74%), followed by OCA1 (4/23 patients, 17%) and OCA4 (2/23 patients, 9%). An OCA2 variant already described in the literature was found recurrently, accounting for 62% of OCA2 alleles. Four new variants were identified, 2 in OCA2, 2 in TYR. One of the new OCA2 variants was a deep intronic variant, NM_000275.3: c.1951+1215G>T, predicted to activate both cryptic acceptor and donor splice sites. RT-PCR analysis on RNA extracted from a patient's blood sample and a minigene study revealed the inclusion of a 77-bp pseudoexon with the appearance of a premature stop codon, leading to loss of function. Altogether a diagnosis was established. This first report of the genotypic spectrum of albinism in a western Sub-Saharan country will constitute the basis for future studies in Mali and countries from this sub-continental region.

P11 - Clonal Hematopoiesis of Indeterminate Potential is associated with increased NETosis

Dufossée Mélody

Clonal hematopoiesis of indeterminate potential (CHIP) result from the acquisition during aging of mutations in hematopoietic stem cells, in the absence of criteria for hematological malignancy. CHIP have been associated with increased mortality, particularly related to the occurrence of cardiovascular events (CVE). CHIP can be found in all blood cell types, including neutrophils, which are the most numerous leukocytes in humans. Neutrophils are able to infiltrate atherosclerotic plaques and to participate in thrombo-inflammatory phenomena, in particular via the emission of Neutrophil Extracellular Traps (NETs). In this study we aim to decipher if neutrophils represent an actor in the pathophysiology of CVE associated with CHIP through the emission of NETs. We studied 81 patients and the presence of CHIP was defined by the detection of at least 1 mutation with a variant allele frequency =2%. Inflammation was assessed by hsCRP and IL1ß levels and atheromatous burden by detection of a >50% carotid stenosis and presence of multi-truncular lesions. Two plasmatic NETs markers were evaluated by ELISA: myeloperoxidase-DNA complex (MPO-DNA) and citrullinated histone H3 (H3-Cit). We also search for increased NETosis caused by CHIP by measuring the percentage of granulocytes forming NETs by immunofluorescence in 2 mouse models of CHIP: adoptive transfer of TET2+/- bone marrow cells in CD45.1.2 mice and a hematopoietic-specific inducible DNMT3A KO in pdzk1-Cre;DNMT3flox/flox mice. 54% of patients were CHIP carriers. The 2 most frequently mutated genes were DNMT3A and TET2. The presence of CHIP was associated with a significant increase in MPO-DNA and H3-Cit plasmatic levels. However, neither the presence of CHIP nor increased NETs biomarkers were associated with increased systemic inflammation, atheromatous burden, or incidence of complications. DNMT3Aflox/flox mice exhibited increased proportion of granulocytes forming NETs although these results were not significant. The impact of TET2 invalidation on the induction of NETosis is currently under investigation.

P12 - Glycerol, abundantly present in mammalian tissues, induces differentiation of trypanosomes into the stumpy-Glyc transmission-competent forms
El Kadri Mohammad

Trypanosoma brucei is an extracellular parasite responsible for African trypanosomiasis, exhibiting a complex life cycle in an insect vector (procyclic forms, PCF) and a mammalian host (bloodstream forms, BSF). Survival in diverse environments requires morphological and metabolic adaptations. BSF trypanosomes were believed to exclusively thrive in mammalian fluids, particularly blood rich in glucose, as their sole carbon source for central carbon metabolism and ATP production. However, recent studies have shown that BSF trypanosomes can grow efficiently in the absence of glucose if supplied with glycerol, and most parasites reside in the extravascular compartment of the skin and adipose tissue, where adipocytes release substantial amounts of glycerol from lipolysis and glycolysis. Hence, we hypothesized that glycerol plays a novel role in African trypanosome biology, with interactions between adipocytes and extravascular trypanosomes potentially granting a selective advantage to the parasites in mammalian hosts. Our findings demonstrate that when glycerol is metabolized, it induces the differentiation of proliferative slender BSF into growth-arrested or slow-growing forms similar to stumpy BSF (forms preadapted to the insect environment) that can efficiently differentiate to the PCF insect stage, implying a crucial role of glycerol in the transmission of the parasite from the mammalian host to the insect vector. It was examined by glycerol kinase and aquaporin null mutants, which failed to differentiate into stumpy BSF in the presence of glycerol. These results support our contention that glycerol, produced by adipocytes, influences trypanosome metabolism, prompting differentiation for transmission to the insect vector.

P13 - ICAM1 expression by the microvasculature impairs capillary perfusion which compromises hind limb ischemia recovery in diabetic mice.
Foussard Ninon

Introduction: Chronic limb-threatening ischemia (CLTI), one of the diabetic complications, is associated with a poor limb prognosis. Its pathophysiology is poorly understood, and therapeutic targets are missing.
Objective: Our objective is to explore the contribution of endothelial dysfunction in the development of CLTI in diabetic mice.
Method: Hind limb ischemia (HLI) was induced by ligation and resection of the femoral artery in C57BL6/J male mice, in which insulin resistance was induced by a high fat diet, and hyperglycemia by low dose streptozotocin administrations one month later.
Results: According to our previous investigations, ischemic foot re-perfusion, assessed via laser doppler perfusion imaging, was significantly reduced in diabetic animals 28 days after HLI surgery was performed (ratio blood flow in the ischemic leg vs non-ischemic leg=0.27±0.09 vs 0.44±0.21 in control mice; P=0.03), even though angiogenesis was identical in both groups. On the contrary, we found that impaired ischemic foot re-perfusion was associated with an increased endothelial cell activation attested by an increased ICAM1 expression (P<0.0001). We then hypothesized that ICAM1, by interacting with white blood cells (WBC), may compromise the perfusion of capillaries with a diameter smaller than a WBC. Accordingly, we found that WBC circulation velocity in the microvasculature was diminished in diabetic mice (P=0.004) and associated with a decreased percentage of capillaries perfusion by BS-1 lectin (P=0.03). With the aim to test whether ICAM1

overexpression may be responsible for impaired ischemic foot re-perfusion, diabetic mice were administered with anti-ICAM1 antibodies or isotype control for 14 days, starting 14 days after HLI surgery was performed. We found that anti-ICAM1 therapy increased WBC circulation velocity within the microvasculature ($P<0.0001$) and the percentage of perfused capillary ($P=0.009$).
Conclusion: Our results demonstrate that ICAM1 overexpression may compromise HLI recovery in diabetic mice by decreasing WBC circulation velocity and impairing capillary perfusion.

P14 - Evaluation of tumor heterogeneity in colorectal cancer and its implication in metastatic process

Galeano-Otero Isabel

Colorectal cancer (CRC) remains the 3rd most diagnosed cancer in Europe. Liver represents the most frequent metastatic site of colorectal cancers and about 50% of patients with colorectal cancer develop this kind of metastases, despite therapeutic advances and new medico-surgical strategies. Consequently, better understanding of the cellular and molecular biology of colon cancer and liver colorectal metastasis are urgently needed. This study was designed to determine the key process and proteins associated to metastatic dissemination in CRC, in order to identify potential biomarkers for accurate prognosis and establishment of personalized therapies. Thereby, transcriptomic (RNAseq) analysis were first performed on 60 pairs of primary colorectal tumors and liver metastases from the same patients and the more significant markers were validated using RT-qPCR and immunostaining. Some of the identified genes were previously reported linked to cancers but never linked to colon cancer. Others genes were specifically dysregulated in metastatic tissues are with unknown function, never reported in cancer. The functional validation of the identified genes in the metastatic process will help not only for patient stratification and prediction of CRC patients that will develop colorectal liver metastasis but also in the potential development of pertinent therapeutic options for each patient.

P15 - AGR2 : A link between ER proteostasis and cancer proliferation

Guilbard Marianne

The endoplasmic reticulum (ER) is the major site for cellular proteostasis regulation. Disruption in ER folding capacity results in altered protein homeostasis. To cope with ER stress, cells activate an adaptive program, the Unfolded Protein Response (UPR) to restore ER homeostasis. However, alteration of the ER proteostasis caused by severe and persistent ER stress results in a sustained UPR activation which is linked to tumor development. Anterior Gradient 2 (AGR2) chaperone is a protein disulfide isomerase, which mediates the formation of disulfide bonds and assists in the quality control of proteins. In cancer, AGR2 promotes cell survival as regulator of UPR by restoring ER proteostasis, and we have demonstrated that AGR2 is also secreted into the tumor microenvironment where it exerts pro-tumoral activities by promoting tumor cell proliferation. However, the precise mechanisms and signaling pathways by which AGR2 promotes tumor cell proliferation and survival still remain unknown.

P16 - The YAP/TAZ/TEAD pathway controls Cytolethal Distending Toxin induced DNA damage and nuclear remodeling in intestinal epithelial cells

Jia Ruxue

We are frequently exposed to infection with genotoxin-producing bacteria, such as the cytolethal distending toxin (CDT), a prevalent heterotrimeric toxin among Gram-negative bacteria. CdtB subunit causes severe DNA damage in host cells and impairs DNA-damage response, leading to genomic instability and accumulation of mutations. The Hippo pathway plays a critical role in the protection of genome stability in response to DNA damage. In the present study, we investigated the effect of CDT on the Hippo and YAP/TAZ/TEAD signaling pathway. *In vitro* experiments were performed on human intestinal epithelial cell lines. Microarray data and western-blot analyses showed a CdtB-dependent regulation of the transcripts and proteins of the core of the Hippo pathway, such as MST1/2 and LATS1/2 kinases, and their transcriptional coactivators, YAP1 and TAZ. Infection of epithelial cells with CDT-producing bacteria is associated with an increased transcriptional activity of the TEAD transcription factors, the final nuclear effectors of the Hippo pathway. This effect was attributed to CdtB subunit following its ectopic expression. Verteporfin, an inhibitor of the YAP1/TAZ interaction with TEADs, reduced the CdtB-induced effects, i.e. increased TEAD transcriptional activity, increased nuclear remodeling, as well as increased polyploidy, DNA damage and repair, confirming the involvement of Hippo signaling pathway in CdtB effects. In addition, colibactin, a genotoxic metabolite produced by Escherichia coli, induced similar effects. Overall, these data show that infection with genotoxin-producing bacteria modulates the Hippo and YAP/TAZ-TEAD signaling pathway to control nuclear remodeling following DNA damage in intestinal epithelial cells.

P17 - Bcl-xL deamidation : a target against platelet aging ?

Kervadec Joann

Deamidation is the post-translationnal modification removing the amino-group of the lateral chain of Asparagine and Glutamine residues. It can occur in virtually any proteins. It is a spontaneous hydrolysis which doesn't require any enzyme. Consequently, deamidation of Asparagines leads to the production of Aspartate and Iso-Aspartate residues, which modifies the global charge of the protein and can alter its folding and function. The occurrence and kinetics of this reaction depend on both intrinsic parameters such as the n+1 residue, the local structure, and extrinsic parameters such as temperature, ionic strength and pH. Our team focuses on the protein Bcl-xL, an anti-apoptotic member of the Bcl-2 family. We discovered that Bcl-xL undergoes a step-wise deamidation on two Asparagines located in its unstructured loop. Deamidated Bcl-xL builds up with time in cells, which we proved instrumental to determine the age of circulating platelets. Platelets are anucleated blood cells playing an essential role in blood clotting. Their lifespan is around 10 days in humans. To overcome thrombocytopenia, a decrease in the platelet count in the blood stream, platelets can be transfused. However, because of their short shelf life, platelet concentrates are not stored for more than 7 days. Platelets respond to apoptosis controlled by Bcl-2 family protein, and survive as long as the pro-apoptotic protein Bak is kept inactive by the anti-apoptotic protein Bcl-xL. The mechanism by which Bcl-xL disengages from Bak is still unknown. We currently investigate whether deamidation is responsible for platelet termination and test whether Bcl-xL deamidation is the mechanism releasing Bak and inducing platelet apoptosis. If this is the case, a direct application will be the screening of compounds that delay Bcl-xL molecular aging and maintain its function in platelet concentrates, thus improving transfusion efficiency.

P18 - Adenovirus spreading and restriction factors in primary bronchial epithelia
Tymchenko Anastasiia

Adenoviruses are considered respiratory viruses infecting the upper respiratory tract and causing mostly mild symptoms like the common cold. The relatively mild disease progression in most cases is owed to good and life-long immune control. Some genotypes are associated with severe respiratory infections coinciding with the release of proinflammatory cytokines. We would like to understand how adenovirus infections spread in bronchial epithelia and what intrinsic mechanisms control the spread and epithelial response. To address this, we established a model of primary bronchial epithelia grown at the air liquid interphase derived from lung brushes of adult vs. child donors.We monitor the adenovirus spread within the epithelia using live cell imaging and fluorescence microscopy at different stages of infection. We measure epithelia integrity together with apical and basolateral virus release over time to understand the infection dynamics between different donors. To identify the epithelial response to infection we monitor cytokine release and the transcriptional response of the epithelia using scRNA-Seq. We found that infection of epithelia progresses from established infection foci. Those foci develop into macroscopic lesions at defined time points after infection. Lesions appear in both age groups and coincide with decrease in transepithelial electrical resistance showing the disruption of the epithelial integrity. Viral spread occurs initially via the apical side of the epithelia, proceeding to the basolateral side once the epithelia is damaged. Interestingly, some donors show a clear delay in lesions appearance, reminiscent of an increased intrinsic resistance to infection progression.

P19 - Characterization of an intriguing protein, BILBO3, in the flagellated pathogen Trypanosoma brucei.
Lambert Chloé

Trypanosoma brucei (T. brucei) is a unicellular, flagellated parasite belonging to the genus of organisms that causes African Human trypanosomiasis (sleeping sickness). Its flagellum exits the cell body through the flagellar pocket (FP). The FP is formed by an invagination of the plasma membrane at the base of the flagellum and is the unique site of endo and exocytosis. The FP is maintained enclosed around the flagellum by the flagellar pocket collar (FPC). The FPC is an essential cytoskeletal structure but its composition, structure and function are poorly known. The team previously identified and characterized the first FPC proteins, BILBO1 and BILBO2. Their N-terminal domains (NTD) are similar in structure with several essential residues that are conserved. We have identified three other proteins with NTDs that share 30% homology with the BILBO1-NTD. Our attention is currently focused on a novel member of the BILBO family called BILBO3. Compared to BILBO1 and BILBO2, BILBO3 has an atypical localization: it is observed at the shank of the Hook Complex and in the basal body region. This intriguing localization prompted me to characterize BILBO3 more profoundly by studying its localization during the cell cycle, its cellular function and its potential protein partners. Using expansion microscopy and BioID, I identified BILBO3 cellular localization and identified several uncharacterized partners. These data will enable us to develop further the FPC-Hook Complex interactome to have a more profound understanding of the roles of these proteins and associated structures, particularly in biogenesis. Keywords : trypanosoma, basal bodies, BILBO3, BioID, ultra-structure expansion microscopy.

P20 - Sema3A/Neuropilin1 pathway drives myeloid cell recruitment and vessel dysmorphia in Glioblastoma microenvironment.

Leboucq Teo

Among primary brain tumors, glioblastoma multiforme (GBM) is the most prevalent high grade glioma. GBM displays highly necrotic, hypoxic and mitotic areas, hallmarks of high grade neoplasms. Current therapy consists of debulking, followed by chemoradiotherapy, resulting in a median survival of 14.6 months. Novel clinical treatment regimens have so far had little impact on GBM patient survival. The field of immunotherapy offers promising new avenues with the example of recent clinical trials targeting Programmed Cell Death protein-1 (PD-1) in order to regulate the immune checkpoint in GBM. Targeting PD-1 aims to release the break on the adaptive immune response against tumour cells, leading to increased recruitement and activation of cytotoxic T cells (CTLs). Despite numerous clinical trials, and novel biological compounds being tested, the poor prognosis for GBM patients remains largely unchanged. The uniquely immune-priviledged microenvironment of the central nervous system proves to be particularly challenging for the efficacy of immunotherapy in GBM. Therefore, the development of new therapies with improved activity at the tumor site will require a deeper understanding of the dynamic tumor micro environment (TME) in GBM. Lately, looking for a novel anti-angiogenic therapy to overcome the resistance observed in response to classical anti-VEGF therapy in gliomas, we evaluated the potential of Sema3A and its receptor Neuropilin1. Surprisingly enough, depletion of Sema3A, either by targeting its expression with shRNA in tumor cells resulted in: i) limiting peripheral macrophages recruitment, ii) blocking the phenotypic switch of macrophages from cytotoxic to tumor supportive, iii) normalizing vasculature allowing optimal chemotherapy delivery within the tumor, and iv) inhibiting glioma progression via enhancing macrophages cytotoxicity. These results suggest that innate immunity importance might have been underestimated to the benefit of adaptative immunity in the quest for effective immunotherapeutic agents. In fact, we identified the crucial role of myeloid cells recruitment and polarization in the organization of glioblastoma stroma, and their deleterious effects on tumor vasculature, promoting immunosuppression, blood vessel dysmorphia, leakage and perfusion defects, limiting chemotherapeutic delivery and immunity derived cytotoxicity.

P21 - FKS resistance mutations affect the composition of the fungal cell wall in Candida by over-activating the Cell Wall Integrity pathway

Lefranc Maxime

The fungal cell wall is essential to maintaining cellular integrity and viability and the cell wall integrity (CWI) pathway is the central MAPK cascade required to trigger an appropriate salvage response under parietal stress. Echinocandins recommended as optimal treatment for invasive candidiasis, are the first class of antifungals that target the fungal cell wall. They inhibit the 1,3-ß-glucan synthase, involved in biosynthesis of the major glucan component of the cell wall. In C. albicans, the main mechanism of echinocandin resistance involves non-synonymous mutations in the FKS1 gene, decreasing the binding affinity of glucan synthase to echinocandins, and among which S645P and R1361G are the most common. In this work, we evaluated the impact of the FKS1 mutations on the composition of the fungal cell wall and on the CWI rescue pathway, using the laboratory model Candida lusitaniae CBS 6936 and genetically engineered isogenic strains carrying the FKS1 mutations at the equivalent position (S638P and R1352G). Previous work has shown that the S638P mutation

is responsible for biochemical cell wall changes, observed by atomic force microscopy and quantified by infrared spectrometry. Our results confirm that FKS1 mutations induce a cell wall remodelling probably via an overactivation of the CWI pathway. FKS1 mutations lead to overexpression of the MKC1 gene, which encodes the kinase Mkc1p of the CWI pathway. Furthermore, the amount of active phosphorylate Mkc1p is increased in FKS1 mutants. Finally, deletion of the MKC1 gene in the FKS1 mutants partially restores sensitivity to echinocandins, suggesting that echinocandin resistance is partly dependent on the CWI pathway. We hypothesise that, like echinocandin drugs, FKS1 resistance mutations compromise the integrity of the fungal cell wall, causing parietal stress for Candida, which in response overactivates the CWI rescue pathway. Thus, FKS1 echinocandin resistance mutations would be not only affinity but also functional mutations.

P22 - Involvement of the transcription factor NRF1 in Alpha 1-Antitrypsin Deficiency mediated liver damage

Lehmann Alexandra

Alpha1-antitrypsin Deficiency (AATD) is a rare genetic disease which can be responsible for liver diseases such as cirrhosis and cancer. This condition is characterized by the retention of AAT mutant proteins in the endoplasmic reticulum (ER) of hepatocytes. The most severe and common disease causing-allele is called Z variant. This mutant is due to a single mutation (E342K) that leads to the retention and the accumulation of Z aggregates into the ER causing cirrhosis and liver cancers. Liver transplantation being the only curative treatment available, a more detailed understanding of the cellular mechanisms of liver injury is required to develop new therapeutic strategies. Thus, we performed omics approaches (proteomic, genomics) and identified the proteasome as potentially involved in AATD liver damage. Further characterization enabled us to demonstrate that the transcription factor NRF1, active when proteasome activity is altered, is up-regulated only in cells expressing the Z variant, suggesting that the Z variant mediates proteasome impairment compensated by NRF1. We next sough to evaluate the impact of NRF1 loss on AATD mediated liver damage. To this aim we are generating, using Crispr/Cas9 technology, NFR1 non-functional cell lines. Clones were validated by Sanger sequencing and qPCR and we were able to demonstrate the lower expression of proteasome subunits in these clones which suggests an inactivation of NRF1. Finally, we evaluated AAT expression (monomer, aggregate and secreted) to assess the potential impact of NRF1 inactivation and we could observe a significant diminution of total AAT in Huh7 Z NRF1 NF cells. Next, we will continue to characterize the modulation of NRF1-proteasome axis in AATD liver disease and explore its modulation as a therapeutic strategy.

P23 - The Role of MTERF3 in Cutaneous Glucose Homeostasis

Ley-Ngardigal Seyta

This study investigates the role of MTERF3, a mitochondrial protein, in skin health and its interaction with glucose homeostasis, especially under the influence of a Western diet characterized by high sugar consumption. Utilizing three models - standard skin cells, genetically modified human skin cells, and reconstructed skin tissues - we explore the effects of MTERF3 on mitochondrial function and skin health in glucotoxic conditions mimicking high sugar intake. The study involves TaqMan PCR for quantifying MTERF3 and genes related to mitochondrial health. We also conduct a comprehensive proteomic analysis to provide a broader view of protein changes and pathways affected by elevated glucose levels. The Simple Western (Wes) technique is employed for analyzing mitochondrial protein responses

to glucose stress. Promoter-reporter assays investigate MTERF3's transcriptional activity in hyperglycemic conditions, spotlighting genes that govern mitochondrial dynamics in skin cells. Seahorse experiments complement these methods by assessing mitochondrial respiratory function and bioenergetics in response to glucose fluctuations. This project aims to unravel how MTERF3 influences skin physiology in high glucose environments, typical of Western diets, emphasizing mitochondrial function. This research has significant implications for understanding dietary impacts on skin health and managing metabolic disorders affecting the skin. The expected outcomes will reveal new mechanisms linking mitochondrial function to glucose homeostasis in cutaneous tissues, contributing to our understanding of skin's metabolic pathologies influenced by diet. This study is conducted in the framework of a CIFRE contract with LVMH RESEARCH, highlighting its practical relevance and industry-academia collaboration.

P24 - Development of an «Intestine-On-chip» to study infections by opportunistic pathogenic yeast Candida with advanced customized optical microscopy.

Lopez-Garcia Fernanda

Conventional *in vitro* biological models (cells in a Petri dish or Transwell inserts) fail to recapitulate the complex physio-biology of the human body. Alternatively, mouse models are now avoided not only because of ethical issues but also because of a lack of overlap between human and rodents (Cunningham, 2002). Organs-on-chips (OOCs) are an alternative to model organ functionality and recapitulate some of their physiological or pathological features *in vitro* (Huh et al., 2010). Even though the two-chamber commercial design of OOC is almost ideal to recapitulate the physiological conditions encountered in the intestine, its operational design intrinsically does not allow to observe real-time events underflow in culture compatible conditions. The overall objective of the project is to develop a new generation of OOCs in conditions that closely mimic the *in vivo* configuration, i.e. allowing the application of external mechanical cues (flow and stretching). The combination of a confocal microscopy module for high-resolution (but slow) fluorescence imaging with an Optical Coherence Tomography (OCT) module for lower (~µm) resolution but fast and label-free acquisition is envisioned. We aim to provide an in-depth investigation of the mechanisms underlying intestinal infection by Candida yeast with the perspective of identifying new routes for therapeutic treatments. The Intestine-on-chip consists of a microfluidic chip with 2 micro-channels separated by a central porous membrane, on either side of which epithelial cells and vascular endothelial cells will be adhered, mimicking the interface of a vascularized human organ. Two lateral vacuum channels allowing the generation of mechanical stretching of the membrane will be included to mimic *in vivo* intestinal cells environment.

P25 - Variants of oculocutaneous albinism 2 OCA2 gene and pathogenicity of exon10 skipping

Mercier Elina

Genetic diagnosis of patients with albinism is essential to adapt patient care and to offer genetic counselling to families. To date, around 30% of patients remain genetically unsolved, with a significant proportion (60%) presenting at least one rare variant of unknown significance (VUS) in one of the 20 known albinism genes. In a previous study, we looked at a series of VUS from patients with suspected oculocutaneous albinism 2 (OCA2) that mapped in or around exon 10 of the OCA2 gene. This exon is sensitive to skipping during splicing

leading, in healthy subjects, to background levels of non-functional transcripts deleted from exon 10. By combining functional approaches (minigene assay, characterisation of transcripts from skin biopsies) we showed that some VUS in/around exon 10 could significantly increase its skipping and result in a defect in functional protein production sufficient to cause pathogenicity. In particular, several intronic and synonymous variants significantly increase exon 10 skipping, enabling us to classify them as pathogenic (Michaud et al., PCMR 2023). We are extending this study to the characterization of sequences that control splicing of OCA2 exon 10. First, we assess the effect of these rare variants when they are in cis with benign variants such as the common c.1065G>A;p.(Ala 355=), a predominant SNP in light-skinned European populations. The identification of a haplotype with an additive effect on the exon 10 skipping will improve prediction tools. Second, we compare the human and murine sequences by minigene assay. Indeed, in mice, exon 10 of Oca2 is not sensitive to skipping. We therefore select the few non-homologous nucleotides between the two species to test their ability to control splicing using minigenes. This should help identifying critical sequences of splicing regulation and facilitate genetic diagnosis.

P26 - Characterization of NRF2 Role in the Development and Therapeutic Resistance of Human Glioblastoma

Moubarak Maya

The nuclear factor erythroid 2-related factor 2 (NRF2) is a master regulator of human antioxidant defenses. However, constitutive NRF2 activation promotes glioblastoma (GB), WHO grade IV glioma, development, and therapeutic resistance. Despite the therapeutic options, glioma stem cells (GSCs) are responsible for the recurrence of GB tumors. In our study, we investigate the role of NRF2 in GB progression using a P3 patient-derived GB 3D model. Our results show that the tendency to reduce P3 sphere-forming capacity is enhanced in NRF2 KO, however, the precise mechanisms underlying its influence on stem cell maintenance and self-renewal require further elucidation. Our study demonstrates that the absence of NRF2 has no discernible impact on P3 cell proliferation under varying oxygen concentrations (21%, 1%, and 0.1% O2). However, a noticeable reduction in the invasive potential of P3 NRF2 knockout spheres is observed in comparison to control counterparts at 21% O2, suggesting a pivotal role for NRF2 in mediating the invasion of P3 spheres. Also, our study points to the involvement of NRF2 in P3 cell metabolism which is to be explored further in terms of the implicated metabolic pathways (LDHs, MCTs...) linked to their invasive capacity. Next, we aim to investigate the role of NRF2 in sensitizing the P3 cells to TMZ treatment using crisper Cas-9 NRF2 KO P3 cells. Therefore, this study may provide novel evidence to improve the outcomes of GB cancer therapy through NRF2-targeted silencing strategies.

P27 - Targeting PI3K/AKT/mTORC1 signalling in gastric cancer stem cells

Nguyen Tra Ly

Gastric cancer (GC) is the 4th leading cause of cancer death worldwide. We identified and characterized cancer stem cells (CSCs) driving tumor initiation and chemoresistance in GC, including a mesenchymal subpopulation of CSCs detected in circulating blood vessels and metastases expressing CD44v3+ as a surface marker. The PI3K/AKT/mTORC1 pathway is an intracellular signalling pathway important in regulating cell growth and cell proliferation, especially in cancer. We have recently identified the upregulation of PI3K/AKT/mTORC1

pathway in CSC sub-populations from GC patient omics data. The aim of this project is to study the role of the PI3K/AKT/mTORC1 signalling in CSCs tumorigenic and invasive properties in GC using two inhibitors of the pathway in combination. The obtained results showed that BKM-120 (PI3K inhibitor) and Rapamycin (mTORC1 inhibitor) have a potential in preventing tumour growth and dissemination on different subpopulations of GCSCs.

P28 - Unraveling vascular and telocytes remodeling in cutaneous infantile hemangiomas using large-volume 3D imaging

Pechtimaldjian Léa

Infantile hemangioma (IH) is the most frequent tumor in newborns, occurring in up to 1 in 10 births. This benign vascular tumor exhibits a fast-growing phase followed by a slow and gradual involution. Ten years ago, propranolol emerged as a remarkably efficient drug to accelerate its regression. While mechanisms of actions remain largely unknown, previous work of the team has demonstrated the key role of a new stromal cell in the pathogenesis and propranolol response: the telocyte (TC). Given the pivotal role of TCs in cellular communication and organization, we hypothesized that TCs could participate in IH involution by inducing a vascular remodeling process, further potentiated by propranolol. In this study, we aim to investigate the vascular organization of IH through a comprehensive architectural analysis of patient resections, classified according to the tumor state and the treatment. Achieving deep and accurate visualization of remodeling necessitates large volume imaging of tissue. However, working on thick samples require tissue-clearing, a technique initially developed for mouse brains. Nonetheless numerous adaptations are required for skin, a highly pigmented and matrix-dense tissue. Accordingly, we have developed an optimized tissue-clearing protocol named Skin-iDISCO+, specifically tailored for skin clearing, which was previously insufficiently addressed. With Skin-iDISCO+ and compatible antibodies, we successfully identified a limited number of tortuous vessels within tumors. Results showed that the reduction of the tumor vascular burden was correlated with vessel straightening. Additionally, we observed distinctive arrangement of TCs around lesional capillaries. They either form sheets or branching meshwork covering vessels. These arrangements appear as being associated with different tumor states and vessel straightening. These findings indicate that natural or propranolol-induced IH involution involves a dynamic TCs reorganization, leading to vascular remodeling. A deeper understanding of propranolol mechanisms holds promise to explore therapeutic prospects for other vascular tumors with unmet clinical needs.

P29 - Rho GTPase nanoclustering ensures the appropriate ordering of cell cycle events

Peyran Landry

Healthy cell proliferation requires the correct ordering of cell cycle events and checkpoints that delay cell cycle progression when problems arise. It is therefore critical to understand how cells control the ordering of cell cycle events, how checkpoints monitor these events and how cells respond to sustained checkpoint activation. We have discovered that the establishment of a polarity axis is a key event controlling the correct ordering of the cell cycle in Saccharomyces cerevisiae. Polarity axis establishment in budding yeast requires activation of the conserved Rho GTPase Cdc42. Defects in polarity trigger a Swe1 (Wee1)-dependent checkpoint that delays mitotic events via inhibitory phosphorylation of Cdk1. Using specific mutations that perturb the recruitment of Cdc42 activators to plasma membrane-localized

nanoclusters, we observe that catastrophic cell cycle defects rapidly ensue. These defects include cell cycle misordering and the accumulation of multinucleate cells, despite robust Swe1 (Wee1)-dependent inhibitory phosphorylation of Cdk1. In searching for signals upstream of Swe1 (Wee1) that may link the polarity machinery to the cell cycle, we found that the activation of this checkpoint involves a novel signal emanating from G1 cyclins. This signal appears to contribute to full Swe1 (Wee1) activity and the protective effect of the checkpoint when polarity defects are encountered. Since G1 cyclins are essential for Cdc42 activation and polarity axis establishment, they would therefore be perfectly positioned to relay problems in polarity establishment back to the cell cycle machinery in order to activate Swe1 (Wee1) until polarity defects are rectified. Collectively, our study illustrates an unexpected mechanism through which cell cycle ordering is controlled to ensure robust, healthy cell proliferation and safeguard cells from unscheduled whole genome duplication.

P30 - Early Impact of the Jak2V617F Mutation on HSCs

Poulet Arthur

MyeloProliferative Neoplasms (MPN) such as Polycythemia Vera (PV), Essential Thrombocythemia (ET), and Primitive MyeloFibrosis (PMF) are haematological malignancies often driven by a single mutation occurring in hematopoietic stem cells (HSCs). The JAK2V617F mutation, predominant in 95% of PV and 60% of ET or PMF patients, initially confers a proliferative advantage to HSCs, leading to clonal expansion and then hyperplasia. The primary objective of this study was to elucidate how a single mutation can lead to three distinct types of blood malignancies. Given the heterogeneity of HSCs , we hypothesized that the nature of malignancy would depend on the specific subtype of HSCs harbouring the mutation. To assess the early impact of the Jak2V617F mutation in HSCs on their self-renewal and differentiation trajectory, we used an inducible lineage tracing mouse model in which the mutation is induced only in a sub-fraction of HSCs (2 to 5%). Four weeks after induction, labelled hematopoietic stem and progenitor progeny were collected for 10x genomics library preparation. Quality control measures were implemented to ensure the quality of the obtained libraries, including cell number assessment and verification of equal contributions from diverse samples. Sequencing data underwent in-depth bioinformatic analysis using an established Seurat workflow. Our preliminary findings indicate a distinct enrichment in the population of Jak2 mutant HSCs within the bone marrow. Moreover, differential expression analyses have revealed overexpression of 2 genes which need to be functionally validated. Deeper bioinformatic analysis are ongoing to identify mutant specific cluster within the HSCs compartment

P31 - Impact of DDR1 on Renal Cell Carcinoma development

Redouté Chloé

In 2018, 350 000 new cases of kidney cancers have occurred in the world. Around 90% of them are Renal Cell Carcinoma (RCC) and the most common subtype is clear cell Renal Cell Carcinoma (ccRCC) which accounts for 75% of all cases. The current ccRCC treatment involves a dual therapy comprising an antiangiogenic agent (TKI or tyrosine kinase inhibitor) and an immune checkpoint inhibitor. TKIs have numerous targets, including the discoidin domain receptor DDR1, a collagen-activated tyrosine kinase receptor. Using TCGA, in ccRCC, DDR1 high expression is correlated with high patient survival, suggesting that DDR1 can inhibit ccRCC development. To study the role of DDR1 in ccRCC development, 2 ccRCC cell lines

called Renca and 786-0 were used. Both cell lines were modified to overexpress DDR1 or knock out for DDR1. Proliferation, cell cycle, migration and collagen invasion were analyzed *in vitro*. In cells overexpressing DDR1, a collagen dependent reduction of proliferation was observed, characterized by an accumulation of cells in G1 phase and a decrease in S phase mainly explained by a trend towards a p21 and p27 expression increase and towards a CDK1 and CDK2 expression decrease. DDR1 overexpression decreased collagen dependent migration of 786-0 cells and tended to reduce migration of Renca cells. Moreover, Snail1 and Zeb2 expressions, two EMT key markers, were decreased in Renca cells. In mice, orthotopic implantation of the ccRCC cell lines overexpressing DDR1 leads to a decrease of tumor development. Taken together, these data show that DDR1 reduces proliferation and migration of ccRCC cells by modulating key proteins of the cells cycle and gene expressions involved in EMT. Consequently, ccRCC development is impaired. As perspective of this work, the mechanisms leading to a decrease of DDR1 expression in ccRCC will be investigated. This could lead to the identification of potential new therapeutic targets

P32 - 3D models for hepatocellular carcinoma research: Investigating the interface between healthy hepatocytes and HCC tumor cells

Rouyer Lucile

Hepatocellular carcinoma (HCC) is a highly heterogeneous cancer that develops over an extended period on a pathological liver. Consequently, the interactions between cancer cells and normal cells constantly evolve throughout the various stages of the disease. These dynamic interfaces represent the battleground where tumor cells encounter the defenses of healthy tissues, including cells and the extracellular matrix. New three-dimensional (3D) models emerge as promising models to study HCC in a more physiopathological context. Our research project focuses on investigating tumor progression specifically at the tumor/healthy tissue interfaces. To address this, we uses a combination of spatial tissue matrix-assisted laser desorption/ionization (MALDI) imaging and Mass spectrometry (LC-MS/MS) analysis on formalin-fixed paraffin-embedded (FFPE) biopsies obtained from proliferative or non-proliferative HCC patients. By identifying the proteome of these interfaces, we aim to uncover potential molecular targets that contribute to the dynamics of these regions. In parallel, we develops 3D models using primary human hepatocytes, HepaRG and HCC tumor cells. We uses a high throughout microfluidic device to produce alginate capsules or tubes containing a mix of healthy and tumor cells or spheroids. We aim to recreate the interface between healthy tissues and the tumor in a confine space delimited by alginate boundaries. The next step is to perform MALDI imaging of our 3D model to see if we recapitulate biopsies architecture to confirm that our HCC 3D model is close to patient and tissue scale. Finally, we want to test target proteins identified combining MALDI imaging and mass spectrometry in our 3D model to gain insights into the mechanisms underlying tumor progression at the interface scale between tumor and healthy tissue.

P33 - Invadosome formation in response to bacterial genotoxins

Saraiva Mariana

We are frequently exposed to bacterial genotoxins, such as Cytolethal Distending Toxin (CDT) and colibactin, produced by bacteria from the microbiota. These genotoxins cause DNA damage and a high degree of ploidy in host cells, well-known risk factors for carcinogenesis, along with stress fiber formation and deep cytoskeleton remodeling. We observed circular F-actin structures following exposure to bacterial genotoxins that may correspond to invadosomes, whose ability to degrade matrices contributes to invasion and metastasis. In this study, we investigated the mechanism of invadosome formation in response to bacterial genotoxins. *In vitro*, the staining of invadosomes' markers in hepatic and intestinal cell lines infected with genotoxin-producing bacteria allowed the confirmation of functional invadosomes. The increase in invadosomes formation was dependent on the CDT and colibactin, as it was not observed in non infected cells and in response to the corresponding mutant strains invalidated for these toxins. Extracellular matrix (ECM) degradation was increased following exposure to these genotoxins. Similar results were observed when using transgenic cell lines expressing the CdtB catalytic subunit of CDT, as well as with DNA-damaging agents (Etoposide and Streptozocin), suggesting that DNA damage leads to invadosome formation and ECM degradation. In response to CdtB, a global kinase activity assay revealed the activation of Src-family kinases, crucial in invadosome formation, that was corroborated using the Src-family kinases inhibitor PP2. Overall, these data show that the genotoxic stress induced by bacterial genotoxins leads to invadosomes formation and ECM degradation, suggesting that chronic and/or repeated exposure to genotoxin-producing bacteria is implicated in cancer progression.

P34 - Role of carbohydrate-binding proteins in controlling glioblastoma stem cell fate and tumorigenesis

Sliusar Myroslava

Carbohydrate-binding proteins (galectins) are lectins known to bind the ß-galactoside sugars. It has been shown that galectins are associated with the development of different types of cancer, including glioblastoma (GBM). The perspective strategy for improving clinical outcomes for patients with glioblastoma is identifying novel targets to eradicate glioblastoma stem cells (GSCs), where galectins can be considered worthy targets for further investigation. To start with, we revealed that Galectin-1, -3, -8, and -9 are highly expressed in the TCGA cohort of patients with glioblastoma and have the most prominent impact on the patient's survival. Surprisingly, patients with simultaneous high expression of galectin genes LGALS1, LGALS3, LGALS8, and LGALS9 had the worst prognosis over other combinations. The analysis of scRNA-seq data showed that Galectin-1 and Galectin-3 are extensively distributed among different types of cells in glioblastoma tumors. Our further experiments with the patient-derived GSCs BTSC73 confirm that the siRNA depletion of LGALS3 leads to a decrease in the number of live cells. The nature of this effect is not related to cell death since we did not detect changes in the number of apoptotic cells by Annexin/PI double staining flow cytometry-based assay. Nevertheless, using EDU staining, we determined that the observed effect is enabled by the reduction of proliferation. In another set of experiments, Western blot analysis revealed that transfection of BTSC73 with siLGALS3 resulted in a decrease in the level of stem cell markers Sox2 and Nestin. To conclude, GAL-1, GAL-3, GAL-8, and GAL-9 can be considered worthy targets for further investigation in

this direction. Regardless of the others, Galectin-3 could be involved in cancer stem cell regulation by controlling proliferation. Gal-3 also affects CSCs by controlling the level of cancer stem cell markers Sox2 and Nestin.

P35 - Characterisation and role of lipid droplet catabolism in glioblastoma

Tessier Cloé

Glioblastoma (GB), the most aggressive primary brain tumors, are characterized by intratumor heterogeneity and a high plasticity of GB cells enabling adaptation and survival under microenvironment pressure. We aim to understand the role of metabolic adaptation in GB aggressiveness, especially of the catabolism of lipid droplets (LD) which is leading to the release of free fatty acid that can be used as membrane components, signaling molecules or source of energy. We show that LD are more present in the core than at the edge of GB in-vitro and in-vivo models. We then hypothesized that degradation of LD is an important mechanism at the border of the tumor. To characterize this catabolic mechanism we inhibited the processes of LD degradation; lipolysis and lipophagy, by targeting the three main lipases: adipose-triglyceride lipase, hormone-sensitive lipase (HSL) and lysosomal acid lipase. This inhibition induces an accumulation of LD, we identify that one of this lipase, HSL, is more involved in lipid droplet degradation in glioblastoma cells. Its specific inhibition accumulates a higher quantity of LD and induces cell death. Interestingly, inhibition of HSL at lower doses is disrupting the mitochondrial membrane potential. To better characterize the metabolic effect, we measured the mitochondrial respiration, and showed that respiration of the cells is decreased by this lipase inhibition. In conclusion, these results highlight the role of LD degradation and more importantly of HSL in GB cell aggressiveness. Our study indicate that HSL activity seems to be crucial for glioblastoma cell respiration and sustain the cell survival. This study could lead to the proposition of HSL as a new interesting target in GB.

P36 - Role of the Striatin3 protein in liver cancers

Tocqueville Camille

The Striatin3 protein is a protein that was first identified as an auto-antigen in a cancer patient. It has since been linked as part of the Striatin family, a family of scaffolding proteins that are a part of the STRIPAK complex. This complex is known to regulate the PP2A phosphatase and thus has a role in multiple signalling pathways i.e. the Hippo pathway or the MAPK cascade. In most cancers, Striatin3 has been reported as an oncogenic protein that turns PP2A from a tumor suppressor into an oncogenic protein. We pointed out that the Striatin3 protein is downregulated in hepatoblastoma (HB). HB is the most common paediatric liver cancer. In this malignant neoplasm, the beta-catenin protein accumulates and increases Wnt signalling due to recurrent activating mutations in the catenin-beta 1 (CTNNB1) gene. We have shown that the depletion of Striatin3 increases beta-catenin expression in CTNNB1-mutated HB-derived HepG2 and Huh6 cells and conversely, the depletion of beta-catenin increases the expression of Striatin3. Similar data was obtained in the wild-type (WT) CTNNB1- hepatocellular carcinoma Huh7 cells. The overexpression of Striatin3 leads to a decrease in proliferation in hepatoblastoma Huh6 cells. Moreover, Striatin3 showed an involvement in the Hippo pathway in addition to the Wnt pathway, thus, in WT CTNNB1 cells (Huh7) only, the activity of the Wnt pathway is decreased when Striatin3 is overexpressed while the Hippo pathway is activated. The YAP/TAZ proteins

being a part of the degradation complex of beta-catenin alongside their own pathway could explain this contrast between WT and mutated beta-catenin cell lines. As mutations in the CTNNB1 gene are the key driver of hepatoblastoma, it is interesting to better understand the role of Striatin3 in order to deepen our understanding of the Wnt pathway and its role in the formation of hepatoblastoma.

P37 - CXCL16, a novel key player of tumor immune microenvironment remodeling in ß-catenin mutated hepatocellular carcinoma

Vaché Justine

The emergence of immunotherapy for the treatment of advanced hepatocellular carcinoma (HCC) has substantially improved overall patient survival. However, accumulating evidence has demonstrated that ?-catenin mutated HCC are devoid of immune infiltrates and resistant to immunotherapy. The immune escape process characterizing such tumors was first confirmed by the assessment of immune cells infiltration in our 3D *in vitro* model of liver tumor cell spheroids. This localized immunosuppression mediated by mutated ß-catenin could be the result of a deregulated production of chemokines, which are key player molecules involved in cancer cells-immune cells communication. In a liver cancer cell model, the depletion of the mutated form of ß-catenin induced an increased CXCL16 expression and secretion, in both 2D and 3D conformations. Conversely, we observed a diminution of this chemokine following the overactivation of the ß-catenin pathway in ß-catenin non-mutated HCC cell models, also in 2D and 3D. In accordance with these results, our global transcriptomic analyses carried out in cohorts of HCC patients revealed that mutated ß-catenin represses the expression of CXCL16. Moreover, the analysis of HCC patient tumor sections also indicated a decrease of its expression in patients presenting ß-catenin mutation. Interestingly, these tumors were also characterized by a fewer infiltration of Natural Killer cells, known to be recruited by CXCL16. Our results identified CXCL16 as novel negative target of ?-catenin and provide new insights into the functioning of HCC tumor immune microenvironment in a context of ß-catenin mutation.

P38 - Functions of DNASE1L3 in the regulation of anti-tumor immune responses

Vasilakou Aliki

Tumor DNA (tDNA) is crucial in the induction of anti-tumor immunity, by stimulating dendritic cells' (DCs) production of type I interferons, which subsequently activate tumor eliminating CD8 T lymphocytes. Chemotherapy (CT)/ radiotherapy (RT) promote the release of tDNA and consequently "boost"" anti-tumor immunity. However, the mechanisms involved in the regulation of the immunostimulatory potential of tDNA remain poorly understood. The endonuclease DNASE1 Like 3 (DNASE1L3) which is produced by DCs, is known to degrade DNA released by dead and dying cells, limiting its ability to activate aberrant immune responses. Given its DC-restricted expression and function in DNA digestion, we investigated the role of DNASE1L3 in cancer. We observed that Dnase1l3 deficiency did not affect mammary tumor growth in spontaneous or transplantable tumor models. However, the absence of DNASE1L3 inhibited the therapeutic efficacy of CTs in both tumor models. We next showed that DNASE1L3 specifically fragments tDNA released by tumor cells in response to CTs and that this fragmented tDNA is more efficient than the unfragmented one to induce human DCs activation *in vitro*. Finally, murine DCs that were deficient for Dnase1l3 were shown to be impaired in their ability to produce inflammatory

cytokines in response to DNA triggering TLR9 stimulation. Altogether, our work suggests that DNASE1L3 is not only disposing of DNA originating from dying cells. In the context of cancer, DNASE1L3 likely processes tDNA released upon CT to make it amenable to stimulate anti-tumor immunity. Therefore, DNASE1L3 may be used in conjunction to pre-existing cancer therapies to enhance their efficacy.

P39 - Inhibition of proprotein convertases activity results in repressed stemness and invasiveness of cancer stem cells in gastric cancer

Zaafour Anissa

Background. Gastric cancer (GC) is the fourth leading cause of cancer-related death worldwide, with most deaths caused by advanced and metastatic disease and limited curative options. Here, we demonstrated the importance of proprotein convertases (PCs) in the malignant and metastatic potential of GC cells through the regulation of the YAP/TAZ/TEAD pathway and epithelial-to-mesenchymal transition (EMT) in cancer stem cells (CSCs).
Methods. The general PC inhibitor, decanoyl-RVKR-chloromethyl-ketone (CMK), was used to suppress PC activity in CSCs from different GC cell lines. Their tumorigenic properties, drug resistance, YAP/TAZ/TEAD pathway activity, and invasive properties were then evaluated. Mouse experiments were performed to investigate the effect on the metastatic potential of the treated cells and the characteristics of the resulting tumors. The prognostic value of PCs in GC patients was also investigated in molecular databases of GC.
Results. Inhibition of PCs activity in CSCs in all GC cell lines reduced tumorsphere formation and growth, drug efflux, EMT phenotype, and invasive properties associated with suppressed YAP/TAZ/TEAD pathway activity *in vitro*. *In vivo*, PC inhibition in GC cells reduced their metastatic spread and growth. Molecular analysis of tumors from GC patients has highlighted the prognostic value of PCs.

Posters out of competition

Impact of Wnt/ROR2 signaling on the organization and function of BBB perivascular fibroblasts

Bats Marie-Lise

The brain has a unique vascular structure: the blood-brain barrier (BBB). Recently, a new cell type has been identified within the perivascular spaces of the BBB: perivascular fibroblasts (PVF), which cover the penetrating arteries. Because of their location, PVF appear to help maintain vascular stability. These cells also produce the extracellular matrix (ECM) essential for vascular homeostasis and stability. Numerous studies have demonstrated the involvement of the Wnt pathway in the establishment and maintenance of the neurovascular unit. Transcriptomic analysis reveals that brain PVFs express all Wnt pathway receptors, including ROR2 receptor. Interestingly, this receptor has also been described as regulating ECM production in tumor cells. We thus hypothesize that perivascular fibroblasts control endothelial cell functions, and that Wnt/ROR2 pathway expressed by FPVs may promote ECM production and thus play a role in BBB stability. Using an *in vitro* co-culture approach, we demonstrate that PVFs reduce endothelial cells permeability, by significantly increasing the expression of CLDN5, encoding Claudin-5, a tight junction protein. We find an activation of the canonical Wnt pathway in PVFs, *in vivo* and *in vitro*, associated with a decrease in ROR2 expression. Using a siRNA-based approach, we show that deletion of ROR2 expression in PVFs leads to a concomitant decrease in MMP9 expression. Conversely, overexpression of ROR2 by fibroblasts, after lentiviral transduction, appears to participate in ECM degradation, notably by decreasing collagens expression and increasing the production of MMP9. Finally, endothelial cell permeability appears to be impaired after treatment with conditioned medium of fibroblasts overexpressing ROR2, via a down-regulation of CLDN5 expression. These results strongly suggest that PVFs play a functional role within the BBB, modulating endothelial permeability. The Wnt/ROR2 pathway appears to be essential in PVFs, notably by controlling both production and degradation of ECM.

Emerging SARS-CoV-2 variants: Intersection of entry routes, antiviral responses and drugs development

Bertinetti Carole

To help better understanding of the SARS-CoV-2 early replication stage we previously setup biophysics, biochemical and cellular models to monitor and quantify the Spike/ACE2 interactions. Computational model as well as biochemical and biophysical monitoring using pulldown, AlphaLISA and biolayer interferometry (BLI) binding assays were developed. We also developed in cellulo transduction assays using SARS-CoV-2 pseudotyped lentiviral vectors (LV) and pulmonary A549-ACE2 cell lines. This allowed us to recapitulate the early replication stage of the infection. Cell imaging systems based on ANCHOR technology was set up to directly monitor the viral genome entry in addition to the Spike/ACE2 interaction in a cellular context. Flow cytometry assay was also developed to quantify this association at the cell surface (1-2). All these systems were used to select drugs targeting the viral entry pathway from different chemical libraries. Our work led thus to the identification of AB-00011778 as a strong lead compound inhibiting SARS-CoV-2 original strain replication as well as circulating variants (EC50 between 0.1 and 0.5 µM). The current project aim to optimize the inhibition capability of the hit by chemical rational design and use it for deciphering the different viral entry routes and their impact on antiviral cell responses.

G1 phase synchronization prevents ON-target megabase-scale rearrangements induced by CRISPR-Cas9

Boutin Julian

The CRISPR-Cas9 system has revolutionized our ability to precisely modify the genome and has led to gene editing in clinical applications. Comprehensive analysis of gene editing products at the targeted cut-site has revealed a complex spectrum of outcomes. A major concern is the potential genotoxicity of DNA double-strand breaks (DSB), which arise from incorrect or ineffective DNA repair and DNA damage response. ON-target genotoxicity is underestimated with standard PCR-based methods and necessitates appropriate and more sensitive detection methods. Here, we present two complementary Fluorescence-Assisted Megabase-scale Rearrangements Detection (FAMReD) systems that enable the detection, quantification, and cell sorting of edited cells with megabase-scale loss of heterozygosity (LOH). These tools offer highly sensitive readouts to decipher the short-term (murine FAMReD) and long-term (human FAMReD) risk and to find solutions to limit it. They reveal rare complex chromosomal rearrangements caused by Cas9-nuclease and show that LOH frequency depends on cell division rate during editing and p53 status. Cell cycle arrest during editing suppresses the occurrence of LOH without compromising editing. These data were confirmed in human stem/progenitor cells, suggesting that clinical trials should consider p53 status and cell proliferation rate during editing to limit this risk by designing safer protocols. In particular, cell cycle blockade by palbociclib could offer opportunities to make nuclease-based gene therapy protocols safer.

Structural basis of the prototype Foamy Virus accessory protein Bet

Calmels Christina

Foamy viruses are endemic retroviruses in animals, and can also infect humans by zoonotic transmission. They are classified as a distinct subfamily than Orthoretroviruses due to many peculiarities amongst wich their non-pathogenicity, an integration in non-coding regions and a very large tropism. This makes Foamy viruses particularly interesting for gene therapy, and potentially oncolytic virotherapy. In order to optimize these therapeutic applications, though, it is necessary to elucidate the molecular mecanisms underlying these characteristics and the relationship between host and spumaretroviruses. In this context, this project aims at characterizing the Bet auxiliary protein, wich exact function and structure remain unknown. Therefore this project focuses on the structural characterization of Bet via X ray cristallography. To study its function during infection, we have first searched for Bet cellular partners and thus, we have identified protein MB21D2, of unknown structure and function. We have developped proteomic approaches to look for Bet and its cellular partner . Structural and functional caracterization of both these proteins will be exposed in this poster.

CXCL10 epithelium-vascular crosstalk is required for endothelium activation following Sars-Cov-2 infection

Chaillot Laura

The vasculature is heavily impacted by Sars-cov-2 infection. Conflicting results exist about the mechanisms by which sars-cov-2 virus acts on the vasculature. The presence of the virus has been reported in endothelial cells in patients' samples. However, the ACE2 receptor is not detected in endothelial cells when analysed by RNA analysis. Furthermore, *in vitro* models that recapitulate the *in vivo* role of the vasculature upon SARS-Cov2 infection do not exist. Thus, it is important to develop more suitable *in vitro* models. We, therefore, investigated the interaction between Sars-Cov-2 and the vasculature by using our previously developed 3D vesseloid model. We firstly compared gene expression by RNA sequencing between a standard 2D culture model and the 3D vesseloid. Endothelium-specific genes are significantly more expressed in the 3D model when compared to 2D. We then assessed whether Sars-Cov-2 directly infects endothelial cells in the vesseloid model. In the absence of ACE2 in endothelial cells, no infection could be documented. When ACE2 is overexpressed in endothelial cells, a low uptake of viral particles in endothelial cells is observed without efficient viral production. We then investigated the indirect effect of Sars-Cov2 infection by maintaining in culture vesseloids with epithelial cells. After infection of epithelial cells, a significant inflammatory response was detected in endothelial cells. Furthermore, we demonstrated that several cytokines are implicated in the crosstalk between epithelial cells and endothelial cells within the vesseloids, the major cytokine is CXCL10, whose regulatory function is currently investigated in depth. Finally, we investigated the clinical relevance of our findings by using blood samples from Sars-CoV-2 infected patients from Bordeaux (Bordeaux, COLCOV collection). We could demonstrate that the patients' cytokines profiles match the *in vitro* finding, and thus support the validity of our analysis.

Mechanism regulating the sub-cellular localisation of a novel RhoGEF isoform

Claverie Gardair Marie-Charlotte

Cytokinesis is essential for the partitioning of cellular content into two daughter cells. It relies on the formation of a contractile ring composed of actin and myosin filaments at the plasma membrane between the two segregated pools of chromatids. The constriction of the ring through myosin activity drive cleavage furrow ingression, generating two physically separated daughter cells with equal genome content. The activity of the small GTPase Rho1, localized at the plasma membrane, is essential for this process, as it promotes actin nucleation and myosin activation. Rho1 is activated by its RhoGEF Pbl-A in Drosophila. Pbl-A enrichment at the equator of the cell via its interaction with the microtubule-associated protein RacGAP50C determines the zone of Rho1 activation and thus the future cleavage site. Our team has identified a novel Pbl isoform, called Pbl-B, expressed at similar levels than Pbl-A in most tissues. In dividing neural stem cells, the two isoforms display distinct localisation and their activities are important for robust asymmetric cytokinesis. However, the regulation of Pbl-B sub-cellular localisation is not known. To adress this question we developed a structure-function approach which allowed us to identify an additionnal Nuclear Localization Signal (NLS) as well as two basic clusters essential for Pbl-B cortical and nuclear localisation. *In vivo* studies confirmed the importance of these residues for the sub-cellular localisation and function of Pbl-B during cell division.

Plateforme d'histopathologie : Prestations proposées

Dugot-Senant Nathalie

Vous voulez :
- Élaborer un projet expérimental
- Prélever et traiter des échantillons
- Mettre en évidence la morphologie de tissus
- Mettre en évidence des protéines d'intérêt

La plateforme d'Histopathologie est un outil diagnostic à l'échelle tissulaire au service de l'innovation et de la recherche. De nombreux projets de recherches sont développés sur cette plateforme et concernent principalement la cancérologie, les maladies métaboliques, les maladies auto-immunes, la neurologie...

Les Prestations proposées :
- Conseils aux utilisateurs pour projets de recherches
- Conseils pratiques pour l'expérimentation et la préparation des échantillons :
    - o Prélèvement
    - o Fixation
    - o Congélation
    - o Coupes paraffine et congelées...
- Prise en charge des échantillons :
    - o partielle
    - o totale dans le cadre d'une collaboration
- Formation des utilisateurs aux différents appareils
- Colorations à façon
- Marquages à façon (mise au point et validation des anticorps)
- Acquisition d'images en fond clair et fluorescence (épifluorescence, microscopie confocale)
- Réalisation de TMA (Tissue Micro Array)

Etude fonctionnelle et localisation de FBXL4

Gavello Fernandez Esther

La mitochondrie est un organite d'un diamètre allant de 0,75µm à 3 µm, composée de deux membranes. L'une d'entre elles possède de grands repliements, les crêtes mitochondriales, où a lieu la respiration cellulaire. Cette fonction en fait un organe central du métabolisme énergétique cellulaire qui est particulièrement régulé. La principale voie en charge de cette fonction, de type post-traductionnel, est l'ubiquitination. Ce processus consiste en l'ajout d'ubiquitine sur le résidu lysine d'une protéine cible. Le proteasome reconnait ce signal et procède à la dégradation. Ce processus biologique tout comme ces acteurs sont partiellement connus. L'ubiquitination se fait par l'action couplée de 3 ubiquitines ligases : E1/2/3. Ici nous allons nous focaliser sur une ubiquitine-ligase E3 : FBXL4. Elle est à l'origine du syndrome 13 de perte d'ADN mitochondrial qui conduit à des encephalomyopathies. Des articles ont montré par une approche biochimique que FBXL4 est une protéine transmembranaire de la membrane externe mitochondriale et qu'elle a pour cible les protéines NIX et BNIP3. L'objectif de notre étude est de caractériser le mode d'action de cette protéine par approche biochimique et sa localisation exacte dans la mitochondrie par microscopie de super résolution. Nos premiers résultats montrent une colocalisation de FBXL4 avec TOM70-UQCR2-NIX par microscopie et nous constatons par western-blot que FBXL4 interfère bien dans la régulation des protéines de la mitophagie.

Effect of Calcium-dependent channels' inhibition on Gastric Cancer Stem Cells Tumorigenic Properties

Genevois Coralie

Stomach cancer is currently the 4th cause of mortality by cancer in the world. Recent work by the research team has identified a rare subpopulation of cells within tumors, called cancer stem cells (CSC), responsible for the initiation, progression, chemoresistance and dissemination of GCs. The team showed that the use of a general calcium flux inhibitor made it possible to sensitize CSCs to current chemotherapy treatments. The two main Ca2+ influx channels of Store-operated calcium channels (SOCs) are the ORAI and TRPC families. The objective of this project was to study the impact of SOCs inhibition on the tumorigenic properties of gastric CSCs *in vitro*. Subpopulations of epithelial-like and mesenchymal-like CSC from different GC cell lines and patients derived tumors were analysed by transcriptomic analysis to study the expression of calcium channels genes. Inhibitors targeting both ORAI1 and TRPC channels (SKF-96365 and YM58483) or ORAI channel only (GSK7975A) were tested on 2 GC lines (MKN45 and MKN74) *in vitro*. Effects of these inhibitors were determined by proliferation assays and immunofluorescence staining on 2D cultures, and on the tumorigenic properties of gastric CSCs by tumorspheres assays in 3D cultures. Transcriptomic analysis showed that ORAI1 was overexpressed in epithelial-like CSCs and TRPC6 was overexpressed in mesenchymal-like CSCs. Proliferation assays showed a dose dependent effect of SKF inhibitor on both cell lines while only a slight effect was observed with the others inhibitors. Tumorspheres assays showed that preventive treatment of cells with inhibitors decreased the number of tumorspheres. However curative treatment of tumorspheres already formed had no effect on their growth. Immunofluorescence analyses of the consequences of inhibitors on the localization of the calcium channels are on going. We demonstrate that SOCs inhibitors have an influence mainly on tumorspheres initiation properties of CSC in GC. Inhibition of both ORAI1 and TRPC channels seems to be more efficient than ORAI1 inhibition alone. A CRISPR-Cas9 strategy will be implemented in order to identify the Ca2+ channels involved in CSC tumor initiation properties.

Vivoptic, une plateforme d'imagerie optique préclinique pour l'évaluation de stratégies diagnostiques et thérapeutiques.

Genevois Coralie

Vivoptic est une plateforme agréée pour l'expérimentation préclinique localisée à l'Institut d'Imagerie Biomédicale de Bordeaux. Labellisée France Life Imaging (FLI), elle offre après formation de l'utilisateur non seulement un accès à des équipements d'imagerie optique pour le petit animal, mais propose aussi des modèles expérimentaux (lignées génétiquement modifiées, modèles *in vivo* tumoraux) ainsi qu'un ensemble de dispositifs thérapeutiques. Cette plateforme de niveau biologique L1 (pas d'agents pathogènes) dispose de salles de chirurgie et de préparation animale totalement équipées (postes d'anesthésie, monitoring (ECG, T°, rythme respiratoire), micro-injecteur, cadre stéréotaxique...). Vivoptic peut également vous aider pour le design de l'expérimentation *in vivo*, vous accompagner le long de votre projet et dans l'analyse des résultats.
1-Vivoptic, une plateforme d'imagerie optique préclinique. L'imagerie optique est largement utilisée dans la recherche sur le cancer. C'est en effet un outil pratique pour évaluer de nouvelles cibles (après marquage fluorescent d'anticorps ou de fragments, d'aptamères,...), pour réaliser un premier criblage des comportements pharmacocinétiques

et des biodistributions de nanoparticules ou tester de nouvelles formulations galéniques. Après une formation initiale, Vivoptic offre un accès aux imageurs optiques pour l'imagerie par bioluminescence et fluorescence de l'échelle cellulaire jusqu'à l'animal entier : - Le Lumina III (Perkin Elmer) pour l'imagerie 2D par bioluminescence, fluorescence et l'analyse spectrale préclinique. Possibilité de multiplexage dans la gamme du visible jusqu'au NIR-I. - Le FMT4000 (Perkin Elmer) est un tomographe de fluorescence moléculaire 3D préclinique (gamme vis-NIR-I) qui permet une quantification absolue (800 nm). - Un échographe (Aixplorer) Clinique/préclinique (mode B, doppler, élastographie) comportant une sonde souris disponible à la plateforme, utile pour développer des stratégies thérapeutiques et la chirurgie guidée par l'image. L'éventail des modalités d'imagerie optique mises à la disposition des utilisateurs de cette plateforme est illustré dans C. Genevois et al. Int. J. Mol. Sci. 2017, 18(12), 2584.
2-Vivoptic, une offre complète pour l'évaluation *in vivo* de vos agents diagnostiques et thérapeutiques ou de votre stratégie thérapeutique innovante. Vivoptic peut vous fournir une bibliothèque de gènes rapporteurs d'imagerie optique (luciférases, protéines fluorescentes NIR), de vecteurs et de lignées cellulaires génétiquement modifiées. Vivoptic propose également des modèles murins immunocompétents et immunodéprimés de tumeurs solides sous-cutanées, orthotopiques et métastasiques spécialement dédiés au suivi par imagerie optique. La génération de nouveaux modèles biologiques adaptés à votre propre projet est également possible.
3-Vivoptic, lieu de partage de dispositifs thérapeutiques précliniques. Des dispositifs thérapeutiques pour les thérapies géniques *in vivo* (électroporation), l'hyperthermie magnétique, les thérapies photodynamiques (PDT), les ultrasons focalisés de haute intensité sont disponibles sur la plateforme. Vivoptic propose aussi un espace pour installer votre propre dispositif thérapeutique préclinique.

Vect'UB : Vectorology expertise at the service of research

Guyonnet-Dupérat Véronique

The vectorology platform is an academic structure for the production of viral particles for gene transfer. The main activities are the production of viral vectors such as lentivirus, AAV and Adenovirus for over-expression of gene or knock-down of gene expression. Lentiviral vectors are tools of choice for gene transfer. They have the qualities of efficiently transducing a large panel of cells including primary stem cells (neurons, retina, HSC). They allow stable and efficient integration of DNA sequences into the cell genome. Adeno-associated virus (AAV) is a versatile viral vector technology that can be used in a wide range of clinical applications in multiple diseases due its unique biological and biophysical properties. The most important step in viral vector production is the proper design of vector. We will help you to find the best construction that will fit to your future experiments. Our platform can assist you in choosing the best viral vectors for your specific application and target cells. Once the choice of viral vector is done, our platform can also help you to design, construct and product the viral vector containing your gene of interest (or shRNA or CRISPR). This service includes viral vector production, concentration, clarification and titration. We have also different ready-to-use viral vector systems (with different pseudotype/serotype and promoters) carrying fluorescent or resistant proteins. The platform offers a large choice of vectors for constitutive or inducible expression and continues to develop new vectors to propose innovative tools. Vect'UB provides also, stable cell line generation and cell immortalization service, manipulations that require a biosafety level # 3. Vect'UB is a

powerful platform that produces more than 400 lots of viruses a year. The power of these tools in gene transfer and its plasticity explain the success of this platform. Do you need to express a specific protein in your cells of interest? Do you need to inhibit or KO specific protein expression in your target cells? The Vect'UB platform can help you!

Role of F-box leucine rich repeat in the mitochondrial metabolism

Lalou Claude

In mammals, about 99% of mitochondrial proteins are synthesized in the cytosol as precursors that are subsequently imported into the organelle. The mitochondrial health and functions rely on an accurate quality control of these imported proteins. By screening a library of shRNA against hundred of E3 ubiquitin ligases, we have identified that several enzymes of F-BOX leucine rich repeat protein family (FBXL) are involved in the mitochondrial ATP production. Here, we show that the E3 ubiquitin ligase F box/leucine-rich-repeat protein 6 (FBXL6) regulates the quality of cytosolically translated mitochondrial proteins. Indeed, we found that FBXL6 binds to chaperones involved in the folding and trafficking of newly synthesized peptide and to ribosomal-associated quality control proteins. Deletion of these interacting partners is sufficient to hamper interactions between FBXL6 and its substrate. Furthermore, we show that cells lacking FBXL6 fail to degrade specifically mistranslated mitochondrial ribosomal proteins. Finally, showing the role of FBXL6-dependent mechanism, FBXL6-knockout (KO) cells display mitochondrial ribosomal protein aggregations, altered mitochondrial metabolism, and inhibited cell cycle in oxidative conditions.

Cell response to HIV-1 infection Role of the BRCA1/1 pathway on the regulation of non-integrated and integrated viral DNA

Lapaillerie Delphine

Retroviral infection triggers cellular responses that participate in the establishment of stable infection and latency. Viral genome Integration requires the delivery of the intasome to the chromosomal insertion locus. The catalysis of integration can then take place followed by post-integrative events such as insertion site DNA repair requiring poorly defined factors. We have previously shown that the DNA homologous repair recombinase RAD51 can modulate the HIV-1 pre-integrative and post-integrative phases. We report here that the infection triggers the formation of RAD51 nuclear foci by a mechanism dependent on the BRCA1/BRCA2 repair pathway. CHiP and imaging approaches indicate that RAD51 is loaded onto viral DNA as soon as it is synthesized during reverse transcription and before integration. Inhibition of this process leads to a decrease in viral infectivity and integration associated with inhibition of reverse transcription. Finally, the study of the stability of the non-integrated viral DNA under the conditions of inhibition of the BRCA1/2 pathway shows an increased persistence. Understanding the processes leading to the recruitment of these repair factors on the viral DNA could help to decipher their role in the fate of the different populations of viral DNA and, thus, in the persistence of the viral genomes in the infected cell and the establishment of latent virus reservoirs.

Identification of autophagy actors involved in the Alpha 1-antitrypsin deficiency
Léon Céline

The main cause of liver disease associated with Alpha 1-antitrypsin deficiency (AATD), a rare genetic disease, is the retention of AAT mutant proteins in the endoplasmic reticulum (ER) of hepatocytes. The most severe and common disease causing-allele is called Z variant. This mutant is due to a single mutation that leads to the retention and the accumulation of Z aggregates into the ER triggering intracellular injury cascade, cell death and chronic liver damage. While liver transplantation is the only curative treatment available, a more detailed understanding of the cellular mechanisms of liver injury is required in order to develop new therapeutic strategies. Based on the literature, the autophagy, and more particularly ER-phagy, is involved in the degradation of Z aggregates. An impairment in this pathway could lead to Z proteotoxicity and then to liver damages. However, the ERphagy pahway involved in Z-AAT degradation is poorly characterized, little is known about all the actors and no translational research cannot be handled to decipher the role of ER-phagy on AATD liver damages. Thus, our goal is to identify and characterize the ER-phagy pathway associated to the Z mutant. To achieve this goal, we developped an assay to follow the ER-phagy related to the Z variant. This assay is based on the tandem-fluorescent reporters containing two fluorescent components: the low pH-resistant red fluorescent protein (RFP) and the low pH-sensitive green fluorescent protein (GFP). These reporters combine red and green fluorescence emission when located outside lysosomes. Upon arrival in the acidic degradative compartments, RFP maintains its fluorescence, whereas the green fluorescence is quenched. This results in red-only emission within lysosomes that can be visualized and quantified by flow cytometry. Next, based on this assay, we will perform an ER-phagy CRISP/CAS9 library screening to identify the key proteins involved in the ER-phagy-mediated Z-AAT aggregates disposal.

Molecular and cellular basis for host chromatin invasion by retroviral genomes
Parissi Vincent

The integration of retroviral genome requires the functional association between the viral integration complex (intasome) and the host chromatin involving multiple interfaces between the integrase, the target DNA and the histone components of the nucleosome. These associations are regulated by cellular factors or the structure of the chromatin surrounding the targeted nucleosome. Our project aims to identify these functional interfaces and to analyze the influence of factors regulating integration. We first characterized the HIV-1 IN-chromatin interactions by biochemical approaches and in a model of chromosomes spreads highlighting an IN intrinsic property of binding to the chromatin and its regulation by its cellular LEDGF/p75 cofactor. We have also shown the importance of both histone tails and the carboxy-terminal domain of IN in this process. Importantly, we demonstrated that the neighboring nucleosomes modulate the functional binding of the intasome to the substrate nucleosome. The use of drugs or mutations targeting these interfaces confirmed that they participate in the efficiency of integration but also in the insertion site selection. Altogether, these suggest that the retroviral IN CTDs act as sensors of the chromatin structure by scanning available histone and DNA interactions participating for the selection of optimal functional interfaces and, thus for efficient genome invasion. References: Benleulmi,M.S., et al., Retrovirology 2017 Matysiak,J. et al., Retrovirology 2017 Mauro,E. et al., NAR., 2019 Lapaillerie,D., et al., NAR, 2021 Mauro et al., mBio 2023. Grants ANRS INterfaces and StructurIN projects FRM INvasIN project

Metabolic Analyses Service
Pinson Benoît

You want... Develop a metabolomic analysis project Measure the metabolite content of your biological samples Interpret your metabolic and/or metabolomic data Be informed about the practical and/or scientific aspects of metabolic data analysis Our service proposes to... Guide you in the design of your metabolic analysis project Provide quantitative and reproducible metabolic data for water-soluble metabolites Assist you in interpreting your metabolic data Train you in the various scientific and technical aspects of metabolic analysis We will acquire new chromatographic devices (u-HPLC and u-HPIC) coupled to a high resolution mass spectrometer (HRMS). Come and discuss all the new services we will offer with these latest generation devices in 2024.

The FACSility flow cytometry platform
Pitard Vincent

The FACSility flow cytometry platform, created in 2005, is one of the 11 platforms of the UAR TBMCore (INSERM 005, CNRS 3427) of the Biological and Medical Sciences Department (SBM) of the University of Bordeaux. It is the only flow cytometry platform in Bordeaux and with cell sorters. In 2023, FACSility gathers (i) three sorters, including one Aurora CS 4 lasers 48 parameters, one Aria IIu 5 lasers 16 fluorescences under microbiological safety cabinet and one Melody 3 lasers 9 fluorescences and (ii) 5 cytometry analysers: one Aurora 5 lasers, 64 parameters, one Fortessa 4 lasers, 16 fluorescences, two CantoII 3 lasers and one Accuri. The facility is proud to offer spectral flow cytometry with the Aurora instruments. In 2022, two engineers (INSERM and University of Bordeaux) provided support to 155 users from 55 research groups belonging to 30 research units for 4354 hours of instrument use. A contractual engineer has been in charge of sorting on the Aria since March. To sum up, this platform has a very high level of use, thanks to a large selection of instruments and responds to all the themes developed by the research groups. 68% of the hours of use come from the SBM department, 11% from the Neurocampus department, 5% from the Health Sciences and Technologies department and the remaining 16% from other departments. Many teams of the Bordeaux scientific community work on cancer and the flow cytometry facility is a crucial tool for their researches. This activity has been structured in recent years at several levels: (i) through the creation of a large INSERM unit (BRIC, 11 teams), (ii) through the ongoing creation of an Impulse Network at the University of Bordeaux called NEWMOON (New Models in Oncology), (iii) through a local interdisciplinary project called Oncosphere Bordeaux and (iv) in a larger regional network strongly supported by the New Aquitaine Region (Oncosphere Regional Research Network). The flow cytometry facility also provides technologies and instruments very useful for the researches in Neurosciences. The IINS, Neurocentre Magendie, IMM, Nutrineuro units of the Neurocampus department are users with a total of 15 research teams. The specificity of the facility is the diversity of researches run on the instruments and the capacity of the staffs to answer any requests or applications compatible with the instruments. The service seems to respond effectively to the demands of researchers in Bordeaux and the region by providing a range of instruments at the level of the best national platforms and by a rigorous organisation and project management. It is completely open to the scientific community, engineers and researchers. It is also an essential tool for scientific animation and training of students/users with a local, national and international influence.

CRISP'edit core facility
Prouzet-Mauleon Valérie

The CRISP'edit core facility, part of the UAR-TBMcore at the University of Bordeaux, provides service to researchers who want to perform genome editing using CRISPR technology. From a basic frameshift knock-out mutation to complex knock-in genetic changes, our dedicated scientific team will work collaboratively with you to design the right CRISPR tools to accelerate your research programs. We can create stable cell lines with a homozygous or heterozygous gene knock-out. Primary cells can also be engineered. More sophisticated genetic modifications such as the removal of specific exons, insertion of tags in a coding sequence or targeted base mutations are also feasible. Used on a larger scale with sgRNA CRISPR libraries, this technology can also be adapted for genetic screening experiments. The CRISP'edit platform provides service according to your needs: we can advise you in the design of your experiment or we can take the experiment from start to finish in-house, for example performing screen design, cell line selection and bioinformatic analysis of screen results

Oncoprot platform Proteomic Platform for Tissue, Cellular and Subcellular analyses
Raymond Anne-Aurélie

The Oncoprot platform is a proteomic analysis platform dedicated to your biological projects (eg. exploratory proteomic expression analysis, interactomics, PTM analysis). Our specific expertise is to combine laser microdissection and mass spectrometry to study the proteome of all types of cellular or tissue structures. Laser microdissection makes possible to isolate a area of interest, a specific population of cells or a cellular compartment from samples fixed in formalin and embedded in paraffin (FFPE). The proteins are then extracted after reversion of the fixation, and are analyzed by mass spectrometry on a latest generation high-resolution device to identify and quantify them. By proteomic profiling, we can use all the deregulated proteins as an identity card of a pathology, which makes it possible to identfify signatures with diagnostic, prognostic values or to predict response to treatments. Thanks to dedicated bioinformatics and integrative biology tools and the intervention of our team's bioinformatician, we can support the biological interpretation of proteomics data and the graphic representation of your results. Contact: anne-aurelie.raymond@inserm.fr

Helicobacter pylori induces pancreatic lesions in a mouse model of gastric carcinogenesis
Sifré Elodie

Gastric cancer, the 4th cause of cancer mortality worldwide, is mainly caused by a chronic infection with the bacterium Helicobacter pylori, which colonizes the stomach lifelong. It induces chronic gastritis, evolving in some cases to intestinal metaplasia, dysplasia and adenocarcinoma. Many studies have tried to correlate Helicobacter infection with disease in extra-gastric digestive organs like the pancreas. It has been reported in H. pylori infected patients that this infection could affect the physiology of the pancreas without colonising it directly. In this study, we evaluated the consequences of mice infection with different strains of gastric Helicobacters on the histopathology of their pancreas. We performed histopathological analysis of HES-stained paraffin-embedded pancreas tissue sections to evaluate fibrosis, inflammation and other lesions. Preliminary results suggest that mice

infected with H. pylori for 12 months developed chronic pancreatitis and fibrosis, known precursor lesions of pancreas cancer. Understanding the impact of H. pylori infection on lesions of extra-gastric organs could help in fine prevent the emergence of other digestive-track related diseases.

TBMCore : Des plateformes technologiques du Département SBM

Turcq Béatrice

TBMCore est une Unité d'appui, de recherche et de développement constituée de 11 Plateformes technologiques. Les plateformes de TBMCore sont destinées aux études biologiques fondamentales et translationnelles à l'échelle tissulaire, cellulaire et moléculaire. Ces plateformes, situées sur le campus de l'Université de Bordeaux, vous offrent un soutien technologique et sont ouvertes à tous les laboratoires de recherche de l'Université de Bordeaux mais également aux laboratoires extérieurs. Au sein de ces plateformes, nous sommes à votre disposition pour vous offrir du conseil, de la mise à disposition de matériels, des services à façon et des formations techniques et scientifiques pour vos projets de recherche. Ces plateformes vous permettent de modifier génétiquement vos modèles d'études (CRISP'edit et Vect'UB), de travailler en milieu confiné L3 (UB'L3), de cultiver vos cellules en différentes conditions de physioxie (CellOxia), de produire des cultures en 3D (VoxCell), d'analyser vos cellules par cytométrie et de les trier (FACSility), de faire de l'immunomarquage et des analyses histopathologiques (Histopathologie), de l'imagerie *in vivo* (Vivoptic), ainsi que l'analyse de l'expression génique (OneCell), d'un contenu protéique (OncoProt) ou métabolique (SAM) sur l'ensemble de vos échantillons biologiques (fluides, cellules, tissus, animaux). Lors de la journée du département, venez découvrir le poster résumant l'ensemble des prestations proposées par les différentes plateformes de TBMCore.

Plateforme de purification et d'analyse des protéines

Velours Christophe

The MFP's "plateforme de purification et d'analyse des protéines" is open to all, our activity ranges from cloning through purification to the biophysical analysis of proteins. Using different chromatographic techniques, we are able to purify your proteins, recombinant or native, by affinity, hydrophobicity, ion exchange chromatography and size exclusion. Biophysical approaches then allow us to determine the molar mass of proteins in solution, their stoichiometry in a complex, their hydrodynamic radius but also their melting temperature (Tm). We therefore have the possibility of studying the oligomeric state of your proteins in solution, the level of aggregate present in a sample, the effect of mutation on their stability and much more! For more info do not hesitate to visit our website www.mfp.cnrs.fr/facility/PAP .

CellOxia Core Facility : Modeling the hypoxic niche

Villacreces Arnaud

While air is composed of 21% O2 (159 mmHg), the physiological O2 concentration in body tissues is much lower. For instance, in murine bone marrow, Spencer et al. measured an average O2 concentration of only 1.8% (13.3 mmHg) in the extravascular environment. In some pathological contexts such as tumors, O2 concentrations are strongly modified leading to drastic effects. The cellular O2 effects are mediated by oxygenases. The most studied are PHDs (Prolyl Hydroxylase Domain) which, in the presence of oxygen, induce the degradation of HIF (Hypoxia Inducible Factors) transcription factors. In physiological and pathological hypoxia, HIFs are responsible for the transcription of several hundreds of genes with various roles in cellular homeostasis (energy metabolism, cytokine synthesis, signalling pathways, epigenetics, etc...). In tissue culture laboratories, the O2 parameter is very often underestimated/misregarded and many cultures/experiments remain performed under so-called normoxic conditions (~21%) which are actually hyperoxic conditions and therefore not physiologically relevant. Hence, some published *in vitro* results unfortunately present an experimental bias, that can compromise their *in vivo* validation. Our platform CellOxia (UAR TBMCore) offers both expertise and equipment to incubate mammalian cells and/or perform experiments under controlled atmospheric conditions (O2, CO2, temperature) to academic and private laboratories. Through its «PAULA» imager (Leica), users are able to monitor their cell cultures without perturbing the culture conditions.

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